African Journal of Pharmacy and Pharmacology

Volume 12 Number 3, 22 January, 2018
ISSN 1996-0816

Academic Journals

ABOUT AJPP

The **African Journal of Pharmacy and Pharmacology (AJPP)** is published weekly (one volume per year) by Academic Journals.

African Journal of Pharmacy and Pharmacology (AJPP) is an open access journal that provides rapid publication (weekly) of articles in all areas of Pharmaceutical Science such as Pharmaceutical Microbiology, Pharmaceutical Raw Material Science, Formulations, Molecular modeling, Health sector Reforms, Drug Delivery, Pharmacokinetics and Pharmacodynamics, Pharmacognosy, Social and Administrative Pharmacy, Pharmaceutics and Pharmaceutical Microbiology, Herbal Medicines research, Pharmaceutical Raw Materials development/utilization, Novel drug delivery systems, Polymer/Cosmetic Science, Food/Drug Interaction, Herbal drugs evaluation, Physical Pharmaceutics, Medication management, Cosmetic Science, pharmaceuticals, pharmacology, pharmaceutical research etc. The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in AJPP are peer-reviewed.

Contact Us

Editorial Office: ajpp@academicjournals.org

Help Desk: helpdesk@academicjournals.org

Website: http://www.academicjournals.org/journal/AJPP

Submit manuscript online http://ms.academicjournals.me/

Editorial Board

African Journal of Pharmacy and Pharmacology

Table of Contents: Volume 12 Number 3 22 January, 2018

ARTICLES

academicJournals

Vol. 12(3), pp. 27-40, 22 January, 2018
DOI: 10.5897/AJPP2017.4878
Article Number: 256B39C55838
ISSN 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Review

Traditional medicinal plants used for the treatment of diabetes in the Sudan: A review

Sakina M. Yagi[1*] and Ahmed I. Yagi[2]

[1]Department of Botany, Faculty of Science, University of Khartoum, Khartoum, Sudan.
[2]Department of Health of Heath Sciences, Khawarizimi International College, United Arab Emirates.

Received 16 December, 2017; Accepted 18 January, 2018

The prevalence of metabolic disorders such as diabetes among population is of increasing concern worldwide. Sudan is a developing country, where several areas frequently depend on folk medicine. Several herbal preparations have been used in folklore practice in Sudan for the management of diabetes with claims asserting their hypoglycemic effect. Basic research relating to these plants are reviewed in this paper with the intention to highlight their therapeutic potential for the treatment of diabetes and promote their regular use in Sudan. Ethnobotanical information was obtained by an assessment of the available literature in electronic data bases with publications describing the medicinal plants used for the treatment of diabetes. In this review paper, different parts of 38 plant species, are described that are used in the Sudanese traditional medicine and belong to 35 genera and 23 families. Thirty three plants have been documented in scientific literature to possess *in vivo* antidiabetic activity and only one was ineffective in lowering blood glucose level, namely *Striga hermonthica.* Many of the plants in the study review have been studied in *in vitro* models (such as α-amylase or α-glucosidase inhibition) in an effort to explain some of their biomedical interaction. The role of isolated bioactive compounds like trigonelline and 3, 5-dicaffeoylquinic acid in diabetes management is also evaluated in the present review. Ten plants original from Sudan have been already used in clinical trials for the treatment of type 2 diabetes. This review provides useful information on the characterization of such herbal medicines that are utilized in the Sudanese traditional medicine for the control of metabolic syndromes such as diabetes.

Key words: Diabetes, Sudan, ethnopharmacology, pharmacology, toxicology.

INTRODUCTION

Diabetes is a metabolic disorder characterized by chronic hyperglycemia which results from insulin deficient secretion or impaired cellular action of the hormone. Insufficient insulin secretion caused by immune destruction of pancreatic β-cells are vital for insulin secretion and consequent development of insulin-

*Corresponding author. E-mail: sakinayagi@gmail.com.

dependent diabetes (Type 1). In case of abnormal insulin cellular action, type-2 diabetes is developed due to a gradual development of insulin resistance and pancreatic β-cell dysfunction. Symptoms are worsen due to obesity and lack of physical activity by the sedentary lifestyle (Gutch et al., 2014).

Diabetes prevalence is on the rise worldwide as a result of accumulating risk factors well pronounced in economically growing nations. An estimated 69% rise is observed for the prevalence of the disease in adults in developing countries versus 20% for adults in developed countries (Shaw et al., 2010). The prevalence rate of diabetes in Sudan ranks the disease high among others that are of raising great concern for the community and for the medical service (Ahmad et al., 2008).

The total population in Sudan is about 34 million, with 70% of it in the Northern parts. Prevalence of type 1 diabetes is estimated at 0.1% among school-age children 7 to 14 years old while type 2 diabetes is estimated at 10.4% among adult population (over 25 year of age) in Northern Sudan (Elamin et al., 1989; Motala et al., 2003).

A complex mechanism involving enzymes and other factors influences the action of insulin in the management of hyperglycemia. Mainstream drugs that are used to control diabetes fall into three main categories. The first category of drugs aims to enhance endogenous insulin availability and includes agents that act on the sulfonyl urea receptors in the pancreas to promote insulin secretion and others that have an impact on the small intestinal mucosal epithelium.

Medicines categorized as group two are directed to potentiate the response to insulin, among them being thiazoline. This group of drugs seems to act as initiator of peroxisomal receptors responsible for regulation of metabolism of carbohydrates, lipids and proteins. The drugs categorized as group three are represented by α-glucosdiase inhibitors and are targeted at reducing the metabolism of complex sugars (Sheehan, 2003; Thompson and Davis, 2017).

Current glycemic medications burden patients due to variable contraindications and interactions with other drugs as well as limited efficacy, limited tolerability and significant side effects arising from their complex mechanisms of action (Moller, 2001; Rotenstein et al., 2012). Existing pharmacotherapy is still far from achieving optimal blood sugar control in such patients, as an effect of a dysfunction in insulin secretion, action, or both (Gupta et al., 2016).

A number of reviews from different countries have highlighted the significance of medicinal plants application for the control of diabetes (Ezuruike and Prieto, 2014). Despite the wide-spread traditional use of these plants for diabetes management in Sudan, scientific support for the safe and effective use of such plants does not exist. The aim of the present work was to collect data relevant to the traditional control of diabetes with plants wellknown for their medicinal effects in Sudan.

Traditional medicinal plants for the control of diabetes

Data about the use of plants in folklore medicine for the control of diabetes relates either with previously conducted ethnobotanical studies or published papers demonstrating the antidiabetic impact of certain plants in Sudan. The experimental evidence of the antidiabetic activity is highlighted in this review, along with biochemical analysis (where available). Considering comprehensiveness and increasing interest in antioxidant activity in relation to antidiabetic effect of plants, the antioxidant activity of these plants are also reviewed (Ezuruike and Prieto, 2014). A literature search of electronic databases on these plants was carried out and for each of the identified plants, synonyms (for plants which were not identified with their accepted names in the original publication), family name, local name, and plant part used are compiled in Table 1. Available pharmacological evidence of these plants for their therapeutic use is also summarized, and most toxicological relevant studies are presented in Table 2.

Experimental evidence concerning medicinal plants and their phytoconstituents that are used in management of diabetes

Acacia nilotica

A dose of 400 mg/kg body weight (b.w) of an aqueous methanol extract of *A. nilotica* pods significantly reduced the levels of blood glucose, the plasma total cholesterol (TC), total triglyceride (TTG), low-density lipids (LDL), the activity of serum glutamate oxaloacetate (GOT) and pyruvate transaminase (GPT) after one month of treatment in diabetic rabbits compared to the untreated diabetic ones. Furthermore, the same dose also significantly increased the plasma high density lipids (HDL) levels of the treated rabbits but not significant effect on creatinine clearance was observed (Ahmad et al., 2008). Hot water extract of *A. nilotica* pods decreased significantly the plasma glucose level of alloxan-induced Albino mice after 1 to 2 h of administration (Abd el-aziz et al., 2013). A similar observation was obtained in Wistar albino rats treated with 400 to 800 mg/kg of aqueous extract of *A. nilotica* pods and 800 mg/kg of ethyl-acetate and *n*-butanol fractionated from aqueous extract after 12 to 18 h of administration (Auwal et al., 2013). Moreover, Tanko et al. (2013) demonstrated that ethyl acetate fraction obtained from the methanolic extract of *A. nilotica* leaves had a remarkable hypoglycemic effect in alloxan-induced diabetic rats after treatment with 50 and 100 mg/kg for 7 to 12 days. Modified lignin extracted from the hardwood of *A. nilotica* exhibited increased glucose binding efficiency as demonstrated by the decreased glucose diffusion and enhanced α-amylase inhibition in

Table 1. Plants used in Sudanese traditional medicine for treatment of diabetes.

S/N	Plant species	Family	Local name	Part used	References
1	*Acacia nilotica* (L.) Willd. ex Delile	Fabacea	Garad	Pod, bark	(Gaber et al. (2013)
2	*Acacia senegal* (L.) Willd.	Fabaceae	Hashab	Fruit	(Hilmi et al. (2014)
3	*Aloe sinkatana* Reynolds	Xanthorrhoeaceae (Aloaceae)	Sabar	Leaf	Gaber et al. (2013)
4	*Allium cepa* L.	Amaryllidaceae	Basal	Bulb	TajEldin et al. (2009)
5	*Allium sativum*	Amaryllidaceae	Toom	Bulb	Ebrahim et al. (2012)
6	*Ambrosia maritima* L.	Asteraceae	Damesisa	Leaf	Yagi et al. (2013)
7	*Ammi visnaga* (L.) Lam.	Apiaceae	Bizrat al khalla	Fruit	Hilmi et al. (2014)
8	*Balanites aegyptiaca* (L.) Del.	Zygophyllaceae	Laloub	Fruit	Gaber et al. (2013)
9	*Bauhinia rufescens* Lam.	Fabaceae	Kulkul	Leaf	El-Ghazali et al. (1997)
10	*Capparis decidua* (Forssk.) Edgew.	Capparaceae	Tundub	Stem	Zia-Ul-Haq et al. (2011)
11	*Catunaregam nilotica* (Stapf) Tirven (Syn. *Randia nilotica* Stapf)	Rubiaceae	Kir Kir	Fruit	Alamin et al. (2015)
12	*Cicer arietinum* L.	Fabaceae	Kabkabe	Seed	Mustafa et al. (2013)
13	*Cinnamomum verum* J. Presl (Syn. *Cinnamomum zeylanicum* Blume)	Lauraceae	Gerfa	Stem bark	Mustafa et al. (2010)
14	*Citrullus colocynthis* (L.) Schrad.	Cucurbitaceae	Hundal	Seed	El-Ghazali et al. (1997)
15	*Cyperus rotundus* L.	Cyperaceae	Sieda	Rhizome	El-Ghazali et al. (1997)
16	*Eucalyptus globulus* Labill.	Myrtaceae	El kafour	Leaf	Houacine et al. (2012)
17	*Faidherbia albida* (Delile) A. Chev. (Syn. *Acacia albida* Delile)	Fabaceae	Haraz	Root bark	Gaber et al. (2013)
18	*Foeniculum vulgare* Mill.	Apiaceae	Shamar	Fruit	Anitha et al. (2014)
19	*Geigeria alata* (Hochst. & Steud. ex DC.) Oliv. & Hiern	Asteraceae	Al Gadad	Root	Hafizur et al. (2012)
20	*Guiera senegalensis* J.F.Gmel.	Combretaceae	Ghubeish	Leaf	Gaber et al. (2013)
21	*Hyphaene thebaica* (L.) Mart.	Arecaceae	Nabag	Epicarp	Gaber et al. (2013)
22	*Khaya senegalensis* (Desr.) A. Juss.	Meliaceae	Mahogany	Stem bark	El-Ghazali et al. (1997)
23	*Kigelia africana* (Lam.) Benth (Syn. *Kigelia pinnata* (Jacq.) DC.)	Bignoniaceae	Um Shutour	Fruit	Priya et al. (2014)
24	*Lupinus termis* Forssk.	Papilionaceae	Turmus	Fruit	Gaber et al. (2013)
25	*Mitragyna inermis* (Willd.) Kuntze (Syn. *Mitragyna africana* (Willd.) Korth., nom. illeg.)	Rubiaceae	Um Gatto	Fruit	Alamin et al. (2015)
26	*Momordica balsamina L.*	Cucurbitaceae	Abu el Efain	Leaf & seed	Houacine et al. (2012)
27	*Nauclea latifolia* Smith	Rubiaceae	Karmadoda	Leaf	Alamin et al. (2015)
28	*Nigella sativa* L.	Ranunculaceae	Al Haba Alsoda	Seed	Hilmi et al. (2014)
29	*Rhynchosia minima* (L.) DC.	Fabaceae	Irg el Dam	Root	EL-Kamali and EL-amir (2010)
30	*Salvia officinalis* L.	Lamiaceae	Maaramya	Leaf	Houacine et al. (2012)
31	*Sclerocarya birrea* (A. Rich.) Hochst	Anacardiaceae	Hommaid	Stem bark	Mariod et al. (2012)
32	*Senna obtusifolia* (L.) H.S. Irwin & Barneby (Syn. *Cassia tora* L.)	Caesalpiniaceae	Kawal	Fermented leaf	EL-Kamali and EL-amir (2010)
33	*Sesamum indicum* L.	Pedaliaceae	Simsim	Seed	Hilmi et al. (2014)
34	*Striga hermonthica* (Delile) Benth.	Orobanchaceae	Al-buda	Whole plant	Alamin et al. (2015)
35	*Tinospora bakis* (A.Rich.) Miers	Menispermaceae	Irg El Haggar	Seed	Alamin et al. (2015)
36	*Trigonella foenum-graecum* L.	Fabaceae	Hilba	Seed	Gaber et al. (2013)
37	*Vangueria madagascariensis* J.F. *Gmel.*	Rubiaceae	Soum Eyown	Root	Musa et al. (2011)
38	*Zygophyllum coccineum* L.	Zygophyllaceae	Tartir	Whole plant	Gaber et al. (2013)

Table 2. Toxicological studies on plants used in Sudanese traditional medicine for treatment of diabetes.

Plant	Interaction/toxicity studies	Refernces
Acacia nilotica	Co-incubation of 0.01% of the extract in Caco-2 cell monolayers decreased the integrity of the monolayer and the secretory transport of CsA indicating possible inhibition of P-gp	Deferme et al. (2003)
Acacia senegal	Toxicity studies of the ethanol extracts of the stem bark revealed that they exhibited no significant toxicity against *Artemia salina.*	Okoro et al. (2012)
Allium sativum	Components of aged garlic extract did not produce significant inhibition of Cytochrome P450 enzymes *in vitro* and in humans	Markowitz et al. (2003) and Greenblatt et al. (2006)
Ambrosia maritima	A single dose of 2000 mg/kg Body weight of the methanolic extract of *A. maritima* was fatal to rats, but a daily dose of 500 and 250 mg/kg b.w was not fatal.	Afaf et al. (2007)
Ammi visnaga	Acute toxicity (LD_{50}) of intraperitoneal administration of aqueous extract of *A. visnaga* fruit in rats was 3.6 g/kg	Jouad et al. (2002)
Bauhinia rufescens	In the brine shrimp lethality assay, ethyl acetate and methanol extracts of the leaves of the plant were found toxic to the *Artemia salina* with IC_{50} values of 0.059 mg/mL and 0.389 mg/mL. While, both the petroleum ether and ethyl acetate extracts of the stem bark were not toxic to the larva.	Muhammad and Sira (2013)
Capparis decidua	Petroleum ether, chloroform, ethyl acetate and butanol extracts were not toxic against brine shrimps and vero cell lines.	Abdalrahman et al. (2016)
Catunaregam nilotica	Aqueous extract of fruits was safe up to a dose of 2000 mg/kg body weight.	Alamin et al. (2015)
Citrullus colocynthis	A single daily dose of alcoholic extract of *C. colocynthis* (50, 100, 200, 400 g/kg) can have toxic effects on liver cells which may induce hepatocyte necrosis and liver fibrosis	Dehghani and Shahin (2006)
Cyperus rotundus	Ethanol extract of rhizomes was safe up to the dose 2000 mg/kg to Wistar rats	Jebasingh et al. (2012)
Eucalyptus globulus	Ethanolic leaf extract have shown moderate brine shrimp lethality with LC_{50} value of 55.95 µg/mL.	Houacine et al. (2012)
Faidherbia albida	Ethanolic stem bark extract of *F. albida* is relatively safe when used sub-acutely in rats.	Oluwakanyinsola et al. (2010)
Guiera senegalensis	Ethanolic leaf extract have shown moderate brine shrimp lethality with LC_{50} value of 26.94 µg/mL	Houacine et al. (2012)
Kigelia africana	Fruits of the plant given to the experimental rats at doses 100, 200 and 400 mg/kg/day orally were toxic but not fatal.	Adam et al. (2013)
Lupinus termis	Lupins contain certain secondary compounds including toxic alkaloids, such as lupinine	Rahma and Narasinga (1984)
Mitragyna inermis	Alkaloid rich extract derived from *M. inermis* induced a strong inhibition of protein synthesis in mammalian cells but did not exhibit mutagenic or genotoxic activity	Traore et al. (2000)
Momordica balsamina	*In vitro* toxicity results raise concern for chronic use	van de Venter et al. (2008)
Nauclea latifolia	An alkaloid rich extract derived from *N. latifolia* could interact *in vitro* with DNA of bacteria and mammalian cells, leading to G2-M cell cycle arrest and heritable DNA-damage, as well as inducing *in vivo* single-strand breaks in liver, kidney and blood cells	Traore et al. (2000)
Rhynchosia minima	The plant is reported as toxic to fish. The seeds are bitter and poisonous and seed extract shows specific agglutinating action on human RBC	Mali and Mahale (2008)

Table 2. Cont'd.

Salvia officinalis	Ethanolic leaf extract have shown moderate brine shrimp lethality with LC_{50} value of 37.21 µg/mL	Houacine et al. (2012)
Sclerocarya birrea	Stem-bark aqueous and methanolic extracts are relatively safe but *in vitro* toxicity test has raised concerns over chronic use of *S. birrea* extracts	Ojewol (2003)
Senna obtusifolia	The leaves can cause marked toxic effects on rats, and the processing of the leaves by fermentation to produce kawal did not alter the toxic activity of the ingredients in the leaves	Yagi et al. (1998)
Sesamum indicum	Sesame oil or its lignans, due specifically to their methylenedioxyphenyl group, could interact with the P450 isozymes and affect the drug metabolisms or dispositions in human	Gokbulut (2010)
Striga hermonthica	Aqueous extract of whole plant was safe up to a dose of 2000 mg/kg body weight	Alamin et al. (2015)
Tinospora bakis	Aqueous extract of seeds was safe up to a dose of 2000 mg/kg body weight.	Alamin et al. (2015)
Trigonella foenum-graecum	Short-term (90 days) and long term (24 week) feeding of fenugreek seeds to rats at levels equivalent to 2 and 4 times the therapeutic dose recommended for humans (25 g/day) produced no toxic effects.	Udayasekhara et al. (1996) and Sharma et al. (1996)

comparison to the controls (Barapatre et al., 2015).

Acacia senegal

Administration of 200 and 400 mg/kg b.w of ethyl acetate extract from the stem bark of *A. senegal* significantly lowered the levels of blood glucose, serum TC, serum TTG, serum LDL, serum urea and creatinine, and increased the serum HDL level in alloxan-induced diabetic albino rats on day 16 after the administration (Batra et al., 2013). Treatment of CCl4-induced acute hepatotoxicity in albino Wistar rats with 400 and 800 mg/kg/day of the hydroalcoholic (70% ethanol) extract of *A. senegal* pods, orally for 7 days, significantly reduced the liver damage and the symptoms of liver injury by restoration of architecture of liver as indicated by lower levels of serum bilirubin and prevention of hepatic damage (Pal et al., 2014). The components extracted by ethanol from the leaves of *A. Senegal* decreased the activity of sucrose enzyme and appeared to support the control of carbohydrate hydrolysis, and consequently reduces the rise of postprandial blood glucose in diabetics (Abdelhady and Youns, 2014).

Aloe sinkatana

The effects of aqueous extracts of *A. sinkatana* leaves on blood glucose and lipid profile in type 2 diabetic patients was evaluated by Gaber et al. (2013). The volunteers of the experimental group received the aqueous extract (500 g/l) at a dose of 5 mL/day. A significant reduction in levels of fasting blood sugar, TTG, TC and LDL and a significant increase in HDL levels was observed.

Allium cepa

A detailed review on the positive antidiabetic activity effect of *A. cepa* in different animal models, and its antioxidant activity as well as clinical studies on diabetic patients was presented by Akash et al. (2014).

Allium sativum

Adminstration of queous extract of *A. sativum* to induced diabetic rats reduced the blood glucose level, total serum lipids and cholesterol (Thomson et al., 2007; Ozougwu and Eyo, 2010; Badole et al., 2013; Thomson et al.,2016).

Ambrosia maritima

Administration of water, 50% ethanolic, ether or petroleum ether extracts of *A. maritima* whole plant to albino rats significantly reduced blood glucose after 1.5 and 2 h, however without significant changes in insulin

levels (Ammar et al., 1993). Alloxan-induced diabetic albino rats treated orally with 28.5 mg/ kg b.w. of aqueous extract of *A. maritima* aerial parts twice/ day showed significant improvement in most of biochemical parameters (levels of fasting blood glucose, serum insulin, total proteins, albumin, globulin, HDL, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, uric acid, serum TC, TTG and LDL) (Helal et al., 2014).

Ammi visnaga

Aqueous extract of *A. visnaga* at the dose of 20 mg/kg b.w significantly reduced blood glucose in induced-streptozotocin diabetic rats after repeated oral administration for nine days (Jouad et al., 2002).

Balanites aegyptiaca

The addition of l0 % whole or extracted pulp of *B. aegyptiaca* fruits instead of starch in the basal diet of alloxan-induced albino rats, for 20 days, caused a significant decrease in serum glucose level and inhibited the activities of serum GOT and GPT (El-Saadany et al., 1986). Aqueous extract of mesocarps of the fruits exhibited a prominent antidiabetic activity when offered orally in streptozotocin-induced diabetic mice (Kamel et al., 1991). Administration of fruits aqueous extract (1.5 g/kg b.w daily for 45 days) in streptozotocin-induced Wistar albino diabetic rats significantly reduced the mean plasma glucose and malondialdehyde levels, and significantly increased the mean plasma insulin, liver-pyruvate kinase, and total antioxidant capacity levels. An obvious increase in the weight of the pancreas and the size of the islets of Langerhans, and improvement in the histoarchitecture were also evident in the treated groups compared to untreated ones (Khalil et al., 2016).The antidiabetic activities of different fruit extracts and fractions of *B. aegyptiaca* were tested in cultured C2C12 skeletal muscle cells and 3T3-L1 adipocytes. An 18 h treatment with 200 µg/mL of the sugars fraction, dichloromethane (E) and ethyl acetate (F) successive extracts increased basal glucose uptake in muscle cells. Only E and F extracts accelerated the triglyceride accumulation in pre-adipocytes undergoing differentiation (Motaal et al., 2012). Dichloromethane and ethyl acetate extracts of the fruit were standardized by high-performance liquid chromatography to contain 0.031 and 0.239% of rutin, and 0.007 and 0.004% of isorhamnetin, respectively (Abdel Motaal et al., 2012). Trigonelline (3-carboxy-1-methyl pyridinium) was identified in the fruits (8 and 13 mg in the peel and pulp respectively) in addition to the flavonoids quercetin, isorhamnetin flavonol and epicatechin (Farag et al., 2015). Saponins, 26-O-beta-D-glucopyranosyl-(25R)-furost-5-ene-3 beta, 22, 26-triol 3-O-[alpha-L-rhamnopyranosyl-(1-2)]-[beta-Dxylopyranosyl-(1--3)]-[alpha-L-rhamnopyranosyl- (1--4)]-beta-D-glucopyranoside and its 22-methyl ether, 26-O-beta-D-glucopyranosyl-(25R)-furost-5-ene-3 beta,22,26-triol 3-O-(2,4-di-O-alpha-L-rhamnopyranosyl)-beta-D glucopyranoside and its methyl ether were also isolated and identified. It was revealed that the individual saponins did not show antidiabetic activity, while their combination resulted in significant activity.

Bauhinia rufescens

The oral administration of 200, 300, and 400 mg/kg b.w methanol extract from the leaves of *B. rufescens* (once a day, for four weeks) significantly lowered the blood glucose levels in alloxan-induced diabetic rats in a dose dependent manner (Aguh et al., 2013).

Catunaregam nilotica

Acute and chronic treatment of streptozotocin-induced diabetes rats with aqueous extracts of *C. nilotica* (Syn. *Randia nilotica*) fruit at 400 mg/kg significantly lowered blood glucose, serum lipid and creatinine levels, and brought back the activity of AST enzyme to normal level. Histopathological studies showed that the aqueous extracts of the plant reinforced the protection of liver (Alamin et al., 2015). Methanolic extracts of leaves, bark and seedcake of *C. nilotica* possess good antioxidant activity and high phenolic content (Mariod et al., 2012).

Capparis decidua

Fruits of *C. decidua* decreased the lipid peroxidation and altered free radical scavenging enzymes such as superoxide dismutase and catalase in erythrocytes, liver, kidney and heart in alloxan induced diabetic rats (Agarwal and Chavan, 1988; Yadav et al., 1997). Moreover, the fruit extract showed satisfactory inhibitory effect on α-amylase and α-glucosidase enzymes, followed by flowers and leaves extracts (Zia-Ul-Haq et al., 2011).

Cicer arietinum

Adminstration of petroleum ether extract (400 mg/kg) of the seed to alloxan-induced diabetic mice reduced significantly the serum glucose level in both acute and subacute studies (Yadav et al., 2009). The seed showed significant diphenylpicrylhydrazyl (DPPH), nitric oxide and hydrogen peroxide activity (Vadnere et al., 2013).

Cinnamomum verum

Administration of 200 mg/kg b.w of cinnamon aqueous extract to alloxan-induced diabetic rats lowered

significantly the levels of fasting blood glucose, TC, HDL, LDL and TG (El-Desoky et al., 2012). Moreover, administration of bark aqueous extract of cinnamon containing 45 and 75% gallic acid equivalents of polyphenol to streptozotocin-induced diabetic rats at 200 mg per kg b.w. for 30 days displayed hypoglycemic and hypolipidimic effects (IM et al., 2014). The bark is rich in volatile oil and polyphenols including rutin, catechin, quercetin, kaempferol and isorhamnetin have been isolated (Yang et al., 2012).

Citrullus colocynthis

Administration of roots aqueous extract (2000 mg/kg) to alloxan-induced diabetic rats showed hypoglycemic effect and improved serum levels of urea and lipid (Agarwal et al., 2012). Moreover, hydroethanol extract (300 mg/kg bw) of the seed reduced significantly the blood glucose level in alloxan-induced diabetic rats (Oryan et al., 2014). Petroleum ether extract (300 and 500 mg/kg bw) of fruit pulp showed significant hypoglycemic effect in streptozotocin-induced diabetes albino rats (Jayaraman et al., 2009).

Cyperus rotundus

The ethanolic extract of *C. rotundus* rhizomes at dose of 250 and 500 mg/kg b.w, for 3 weeks, revealed significant antidiabetic activity and resulted in improvement of body weight and reduction in the levels of biochemical parameters such as GPT, GOT, TC and TTG in streptozotocin-induced diabetic mice (Singh et al., 2015).

Eucalyptus globulus

Administration of leaf aqueous extract of *E. globulus* at a dose of 150 mg/kg b. w decreased the blood glucose and lipid levels in alloxan induced diabetic rats (Patra et al., 2009). Aqueous ethanolic leaf extract at a dose of 400 mg/kg b.w reduced also the blood glucose level in glucose loaded rats (Houacine et al., 2012). Incorporation of *E. globulus* leaf in diet (20 g/kg) and drinking water (2.5 g/L) had hypoglycemic effect and reduced oxidative stress in streptozotocin-induced diabetic rats (Nakhaee et al., 2009).

Faidherbia albida

The administration of an aqueous extract from the stem bark of *F. albida* at dose 125 to 500 mg/kg b.w to alloxan-induced diabetic rats decreased significantly the fasting blood glucose level in a dose dependent manner and ameliorated the serum markers of the liver, feed and fluid intake, body weight and packed cell volume (Umar et al.,2014).

Geigeria alata

Diabetic rats orally treated with 250 mg/kg of *G. alata* root aqueous methanolic extract for 2 h (acute) appeared to have significantly lower blood glucose levels after 120 min. Constant treatment for 14 days of diabetic rats with 250 mg/kg of *G. alata* extract resulted in a significant decrease in blood glucose level (7.34±0.33 mmol/l) closer to that of nondiabetic rats. At the same time, it significantly decreased serum TTG levels, increased serum insulin levels, improved β-cell function, and the antioxidant status. *G. alata* also showed strong antioxidant and α-glucosidase inhibitory activities in *in vitro* assays (Hafizur et al., 2012).

Guiera senegalensis

A dose-dependent significant reduction in blood glucose levels which was more remarkable at the dose of 400 mg/kg was observed after the application of *G. senegalensis* leaves ethanolic extract (Houacine et al., 2012).

Hyphaene thebaica

Oral administration of aqueous extract of *H. thebaica* mesocarp experimentally caused a significant decrease in blood glucose level in Wistar albino rats, at 12 to 18 h post administration (Auwal et al., 2012). Aqueous extract improved glucose and insulin tolerance, and significantly lowered blood glycosylated hemoglobin levels. Chrysoeriol and 7-O-β-D-galactopyranosyl(1→2)-α-L-arabinofuranoside, which were isolated in the aqueous extract reduced significantly AST and ALT levels of liver and improved the kidney function (Salib et al., 2013).

Khaya senegalensis

The antidiabetic activity of *K. senegalensis* butanol fraction of the root ethanolic extract in type 2 diabetes model of rats was examined by Ibrahim and Islam (2014). The orally administered extract, at 300 mg/kg b.w, significantly reduced blood glucose level, improved oral glucose tolerance ability and β-cell function (HOMA-β), decreased insulin resistance (HOMA-IR), stimulated hepatic glycogen synthesis, ameliorated serum lipids alterations and prevented hepatic and renal damages compared to untreated diabetic rats. Additionally, the fraction tended to improve weight gain, decrease feed and fluid intake, stimulate insulin secretion and lower

serum fructosamine concentrations. Polyphenolic compounds such as catechin, rutin and procyanidins with significant antioxidant activities were also identified in different parts of the plant (Atawodi et al., 2009).

Kigelia africana

In streptozotocin-induced diabetic rats, daily administration of the defatted methanolic extract of *K. africana* flower at the doses of 250 and 500 mg/kg b.w for 21 days reduced significantly the blood glucose and the TC and TTG levels as well (Kumar et al., 2012). Similarly, methanolic extract from the leaves was found to significantly decrease ($P<0.01$) serum glucose level in alloxan-induced diabetic rats after the 21 days of oral treatment (Priya et al., 2014). The ethanolic extract, together with compounds catalpol, specioside and minecoside (10 µM) isolated from the *n*-butanol fraction exhibited significant stimulation of GLUT4 translocation to cell surface from intracellular compartments (Khan et al., 2012). Acetone, ethanol, chloroform, and water extracts of the leaves caused a significant α-amylase inhibitory effect (Dhriti et al., 2014). The root, stem bark, fruit and leaves were found to possess antioxidant activity (Atolani et al., 2011; Sikder et al., 2011; Agyare et al., 2013; Akanni et al., 2014).

Lupinus termis

A dose (75 mg/100 g b.w) of aqueous suspension from *L. albus* orally administered daily to alloxan-diabetic rats restore the changes in the levels of glucose, urea, creatinine and bilirubin and the enzymic activities of AST, ALT and lactate dehydrogenase (LDH) to their normal levels after 4 weeks of treatment (Mansour et al., 2002). In contrast, Sewani-Rusike et al. (2015) reported that the use of *L. albus* may not be effective in treating hyperglycaemia in type 1 diabetes but effective for treating diabetes induced dyslipidemia. They found that *L. albus* demonstrated significant hypoglycaemic effects in normal rats but not in diabetic rats after acute and long term treatment. Normal treated rats showed higher insulin levels compared to normal controls but insulin remained very low in diabetic rats. However, *L. albus* was effective in reducing atherogenic lipid levels.

Mitragyna inremis

Oral administration of aqueous extracts from *M. inremis* fruits at the level of 400 mg/kg to streptozotocin-induced diabetes rats, for 14 days, resulted in a significant antihyperglycemic effect and have the capacity to correct the metabolic disturbances associated with diabetes. Histopathological studies showed that the aqueous extracts of the plant reinforced the protection of liver (Alamin et al., 2015).

Momordica balsamina

Aqueous extract of *M. balsamina* seeds at the level of 500 mg/kg b.w dose caused a significant increase in the blood glucose levels of streptozotocin-induced diabetic rats. Furthermore, after three weeks of treatment of the diabetic animals with the aqueous extract (500 mg/kg b.w) blood sugar level was significantly higher compared to untreated diabetic rats; at the same time, lipid profile and body weight were improved (Bhardwaj et al., 2010). Moreover, aqueous and organic extracts of *M. balsamina* was screened against chang liver, C2C12 muscle and 3T3-L1 adipose cells using a glucose utilization assay. Results showed that *M. balsamina* extracts were active in myocytes and stimulated glucose utilisation in hepatocytes (van de Venter et al., 2008).

Nauclea latifolia

Aqueous leaves extracts of *N. latifolia* at the level of 200 mg/kg b.w significantly lowered glucose levels of the alloxan-induced diabetic rats within 4 h (Gidado et al., 2005). Moreover, the aqueous and ethanolic extracts significantly lowered the fasting blood glucose levels of the streptozotocin-diabetic Wistar rats in a dose-dependent manner after 1-6 h of administration (Gidado et al., 2008). The same results were observed when ethanolic extracts (100, 200 and 400mg/kg b.w) of the leaves were provided orally for 45 days to streptozotocin-induced diabetic rats (Abubakar et al., 2009). Significant reduction was found in the fasting blood glucose, lipid profile (TG and LDL) levels in diabetic rats administered 150 and 300 mg/kg b. w. of n-hexane and methanolic leaves fractions of *N. latifolia* (Effiong et al., 2014). Treatment of Swiss albino mice with 200 mg/kg b.w of ethanolic extract of leaves, twice a day for 21 days, decreased significantly blood glucose in diabetic animals and caused significant decrease ($p<0.05$) in TC, LDL level and ALT and AST activities (Sylvester and Dan, 2015).

Nigella sativa

Several studies demonstrated the hypoglycemic effect of *N. sativa* seed (Benhaddou-Andaloussi et al., 2011; Sathiavelu et al., 2013; Ikram and Hussain, 2014; El Rabey et al., 2017). The seed was shown to ameliorate biochemical and histopathological changes caused by diabetes, decrease oxidative stress, elevate level of insulin, reduce resistance of insulin and hepatic gluconeogenesis, enhance renewal of ß-cells of islets of

Langerhans and create direct insulin-like effects at the cellular and molecular levels in various organs. Seed volatile oil and thymoquinone were found to possess the highest antidiabetic activity (Bamosa, 2015).

Salvia officinalis

The hypoglycemic effect of the aqueous ethanolic extract of *S. officinalis* leaves at the dose of 200 to 400 mg/kg is revealed as a dose-dependent significant reduction of blood glucose levels (Houacine et al., 2012).

Sclerocarya birrea

Following acute treatment, relatively moderate to high doses of *S. birrea* stem-bark aqueous extract (25 to 800 mg/kg b.w) induced a dose-dependent, significant reduction in the blood glucose concentrations of fasted streptozotocin-treated diabetic rats (Ojewole, 2003). Results from male Wistar rats subjected to oral load of glucose (4g/kg) after receiving a dose of 35 mg/kg of aqueous extracts of fresh leaves and barks of *S. birrea* showed that the extracts caused significant antihypergliycemic effects after 2 and 4 h (François et al., 2014). Aqueous and methanolic extracts of the stem bark inhibited the activities of α-amylase and α-glucosidase in a concentration dependent manner. Both extracts possess antioxidant activity, with the methanolic extracts displaying the strongest free radical scavenging capacity. Extracts also significantly increased glucose uptake in C2C12 myotubes, 3T3-L1 adipocytes and HepG2 hepatocarcinoma cells. However, insulin secretion from RIN-m5F cells was not affected (Mousinho et al., 2013). Crude *S. birrea* stem bark methanolic and acetone extracts inhibited human urinary α-amylase more potently than acarbose. Crude hexane extract displayed a strong inhibition of α-glucosidase and weak inhibition of α-amylase. Furthermore, the hexane extract significantly suppressed the rise in postprandial glucose level after oral administration of sucrose but failed to induce similar effects after oral administration of starch and glucose in both normal and diabetic rats (Mogale et al., 2011).

Sesamum indicum

Treatment of streptozotocin-induced diabetic rats with 500 mg/kg b.w ethanolic extract of *S. indicum* seeds for 8 weeks increased significantly the blood glucose and glycosylated hemoglobin levels but decreased significantly the serum insulin and hemoglobin levels. The liver glycogen level was significantly decreased in diabetic rats closer to normal revealing its potential effect to control hyperglycemia (Bhuvaneswari and Krishnakumari, 2012). Alloxan-induced diabetic rats provided with 10% and 20% seeds either raw or roasted as supplemented diet had significantly ($p<0.05$) lower levels of blood glucose, lipids and some serum enzymes (Akanya et al., 2015). Takeuchi et al. (2001) found that hot-water extract from defatted sesame seed and its methanolic fraction had a reductive effect on the plasma glucose concentration of KK-Ay mice, and this effect is suggested to have been caused by the delayed glucose absorption. Amutha and Godavari (2016) demonstrated that *S. indicum* can be used to reduce the postprandial hyperglycemia by inhibiting carbohydrates metabolizing enzymes α- amylase and α- glucosidase, and also to combat the free radicals due to its antioxidant activity. It has been reported that sesame seeds can improve oxidative status due to the activities of their contents including sesamin, sesamolin, sesamol, and sesame (Wichitsranoi et al., 2011).

Striga hermonthica

Daily oral administration of *S. hermonthica* whole plant aqueous extract (400 mg/kg b.w) to streptozotocin-induced diabetic rats for 14 days appeared to increase the blood glucose level, and did not improve the levels of TC, LDL, HDL, urea and blood urea nitrogen indicating that it has no antihyperglycemic effect (Alamin et al., 2015). Kiendrebeogo et al. (2005) found that the aqueous extract of *S. hermonthica* whole plant possessed antioxidant activity and they suggested that the isolated luteolin could be responsible for this activity.

Tinospora bakis

Acute and chronic treatment of streptozotocin-induced diabetes rats with aqueous extracts of *T. bakis* seeds at 400 mg/kg significantly lowered blood glucose levels, and had the capacity to correct the metabolic disturbances associated with diabetes. Histopathological studies showed that the aqueous extracts of the plant reinforced the healing of liver (Alamin et al., 2015).

Trigonella foenum-graecum

Animal's standard diet supplemented with seeds of *T. foenum-graecum* (5%) for 30 days to alloxan induced diabetic rats significantly decreased the levels of glucose, TG, TC and LDL-CH and increased the level of HDL-CH. Also it reduced the oxidative stress by improving the superoxide dismutase, catalase and glutathione peroxidase activities both in serum and in pancreas homogenate (Beji et al. 2016). Aministration of *T. foenum-graecum* seeds (2.5 and 5 g) for 4 weeks to Tunisian type 2 diabetic patients, improved blood glucose

level in dose-dependent and the dose of 5 g reduced significantly TC and TG levels and serum α-amylase activity (Khlifi et al., 2016).

Zygophyllum coccineum

A dose of 1.5 mL of aqueous suspension of *Z. coccineum* herb/100 g b. w (equivalent to 75 mg/100 g b.w), orally administered daily to alloxan-diabetic rats for 4 weeks, restored significantly (P<0.05) the changes at the levels of glucose, urea, creatinine and bilirubin and the activities of AST, ALT, LDH and alkaline phosphatase enzymes in plasma, liver and testes (Mansour et al., 2002). Moreover, 1.5 mL of water soluble extract/kg b.w of the herb, administered orally to alloxan-induced diabetic rats daily for 4 weeks, significantly decreased the blood glucose level and the activity of cytochrome P450, NADPH-cytochrome C reductase, aryl hydrocarbon (benzo(a)pyrene) hydroxylase (AHH), N-nitrosdimethylamine N-demethylase I (NDMA-dI), NADPH-cytochrome C reductase, and detoxified by glutathione S-transferase (GST) and glutathione (GSH) enzymes in the liver of diabetic rats (Sheweita et al., 2002). The leaves were found to possess antioxidant activity (El-Shora et al., 2016). Various compounds from the leaves of *Z. coccineum* were identified by gas chromatography–mass spectrometry (GC/MS) like 1-nonadecene, 9-octadecenoic acid, 2-methyl propanoic acid, β-sitosteol, tricosane and tetracosane, Stigmast-5-en-3-ol, docosene, 1-eicosanol, hexacosane, heptacosane, nonacosane, 6-Ethyl-5-hydroxy-2,3,7-trimethoxynaphthoquinone and pentacosane (El-Shora et al., 2016).

DISCUSSION

Sudan is a developing country that frequently depends on folk medicine in all areas of the country. Several herbal preparations have been used in folklore practice for the management of diabetes with claims asserting their hypoglycemic effect. In this paper, an effort was made to refer to the different parts of 38 plant species that are used in the Sudanese traditional medicine (Table 1). Interestingly, some of these plants have already been reported in previous studies originated from other countries like Algeria (Houacine et al., 2012), Iran (Mikaili et al., 2013), Egypt (Helal et al., 2014), India (Singh et al., 2015), Nigeria (Auwal et al., 2012) and Saudi Arabia (Bamosa, 2010). The reviewed plants have been evaluated, in *in vivo* experiments with diabetic animals that were induced either by alloxan or streptozotocin (Fröde and Medeiros, 2008) in addition to genetically mutated *in vivo* models such as KK-Ay mice. Ten of the characterized plants (*Acacia nilotica, Catunaregam nilotica, Cicer arietinum, Cinnamomum verum, Geigeria alata, Guiera senegalensis, Khaya senegalensis, Mitragyna inremis, Momordica balsamina* and *Tinospora bakis*) tested effective in animal models for their antidiabetic potential from samples collected from Sudan.

In vitro pharmacological evidence

From the 38 plants reviewed in this paper, only four of them were not tested for hypoglycaemic activity, either *in vivo* or *in vitro*. Only one from the 34 plant species was ineffective in lowering blood glucose level, namely *Striga hermonthica* (Alamin et al., 2015), suggesting lack of antidiabetic effect. Ezuruike and Prieto (2014) reported that the absence of an *in vivo* antihyperglycemic effect of some plants would not be a reason to stop their use as antidiabetics, since they may used in multicomponent preparations because of their benefits in co-morbid conditions or possibly be the foundation for comprehensive control of the disease and consequent complications. In fact, components aqueously extracted from *S. hermonthica* whole plant reduced the TG level, improved several liver parameters (reduced ALT activity) and possessed high antioxidant activity (Alamin et al., 2015; Kiendrebeogo et al., 2005). Many of the plants described in the present review have been studied in *in vitro* models that could possibly explain some of their mechanisms of action. Information on the mechanism of action would be an important element in implementing a therapeutic plan for diabetes, considering the likely benefit of the synergy of medicinal plants (Ezuruike and Prieto, 2014). Four plants (*Acacia nilotica, Capparis decidua, Geigeria alata* and *Sclerocarya birrea*) have inhibitory effects against either α-amylase or α-glucosidase enzymes; Seven plants (*Ambrosia maritima. Balanites aegyptiaca, Geigeria alata, Hyphaene thebaica, Khaya senegalensis, Sclerocarya birrea* and *Sesamum indicum*) induce secretion of insulin from ß-cells of the pancrease; five plants (*Balanites aegyptiaca, Cinnamomun verum, Kigelia africana, Momordica balsamina* and *Trigonella foenum-graecum* enhance glucose absorption in muscles or liver or increase GLUT4 gene expression leading to enhanced glucose absorption by muscle and fat tissue and one plant decrease the activity of sucrose enzyme and offer a support to control carbohydrate hydrolysis in diabetic disease.

Bioactive compounds

A number of active compounds have been identified from the plants in this review paper but their role in diabetes management was not proved for most of them. However, trigonelline (3-carboxy-1-methyl pyridinium) was identified in *Balanites aegyptiaca* fruits (8 and 13 mg in the peel and pulp respectively) by Farag et al. (2015). Its discovery provides novel insight into the balanite fruits

antidiabetic properties as the compound is known for a pronounced hypoglycemic effect (Farag et al., 2015). More recently, 3, 5-dicaffeoylquinic acid was found to be the dominant acylquinic acid in Geigeria *alata* roots (25.96±2.08 mg/g dry weight) and ameliorated significantly ($P < 0.05$) the blood glucose and liver biochemical parameters in streptozotocin-induced (40 mg/kg, i.p.) diabetic normotensive Wistar rats and spontaneously hypertensive rats (Simeonova et al., 2016).

Clinical studies

Clinical evaluation, involving human subjects, of biologically active plants is necessary towards the progress of incorporation of medicinal plant products in the health service system (Ezuruike and Prieto, 2014). In this review, 10 plants sourced from Sudan were subjected to clinical trials in Type 2 diabetic patients and results showed that the order of effectiveness of the aqueous extracts of the studied plants to lower fasting blood sugar level was *Lupinus albus* > *Balanites aegyptiaca* > *Allium Sativum* > *Allium cepa* > *Guiera senegalensis* > *Aloe sinkatana* > *Hyphaene thebaica* > *Trigonella foenum-graecum* (Gaber et al., 2013). Capsules containing *Nigella sativa* seeds were administered orally to human volunteers, in Saudi Arabia, in a dose of 1, 2 and 3 g/day for three months. The dose of 2 g/day caused significant reduction in fasting blood glucose levels, while β-cell function was increased after 12 weeks of treatment (Bamosa, 2010).

Toxicological evidence

Assessment of the safety and toxicity profile of herbal medicine is essential to ensure its therapeutic potential. A summary of the studies that describe the toxicological effects of medicinal plants is presented in Table 2. It has been noted that the majority of the investigations corresponded mainly to the determination of acute toxicity and safe dose and included very limited information concerning toxicological and herb–drug interactions. However, some of the plants listed in Table 2, like *Allium cepa, A. sativum, Cinnamomun verum* and *Trigonella foenum-graecum* are actually consumed frequently in Sudan and other countries, and are usually perceived as safe. However, Zaid et al. (2010) reported that garlic and onion bulbs share many similar active compounds (for example, allyl propyl and diallyl sulfide) and decrease blood glucose levels also by normalizing liver hexokinase and glucose-6-phosphatase activities and increase insulin secretion from the pancreas but excessive consumption of these two bulbous plants might lead to harmful effects. *T. foenum-graecum* and *C. verum* exhibited cytototoxic effects at concentrations higher than 500 µg/mL (Kadan et al., 2013). Moreover, consumers are usually aware of possible health hazards occurring after consumption of certain plants and the necessity of their proper process to remove toxicants before utilization. For example, the toxic lupinine found in Lupins (*Lupinus termis*) is removed through debittering process, including soaking in water and daily replacement of water until bitterness disappears before the seeds could be safely consumed. Although, people in Sudan and other African countries consume kawal (fermented fresh leaves of *Senna obtusifolia*), studies have shown that fermentation has not altered the toxic activity of the ingredients in the leaves (Yagi et al., 1998). Thus, toxicological evaluation of medicinal plants is equally significant as their evaluation for efficacy and there is an urgent need for a vibrant pharmacovigilance system to ensure their use in therapeutic management (Shaw et al., 2012).

Conclusion

The quest for control of diabetes has led to an increasing research at different fronts, among which is medicinal plants. Given the observation of an increasing use of medicinal plants for diabetes in Sudan, this necessitates validation of efficacy and safety. *In vitro* experiments are carried out to ascertain the mechanism of action of medicinal plants. The hypoglycemic effect of certain plants arises as a side effect of their *in vivo* toxicity (Marles and Farnsworth, 1995). However, a risk may be posed by the fact that such hypoglycemic effect is probably partially exhibited via an unfavourable physical mechanism overriding a physiological one. As for the validation of experiments, ethical considerations concerning animal use are increased (Festing and Wilkinson, 2007), and therefore the use of non-animal models should be seriously considered. A number of standardization measures, such as reference pharmacopoeial monographs, are necessary to assert the medicinal value of these herbal medicines as reliable and therapeutically effective. Case studies involving standardized medicinal plant products should be carried out in order to validate the usefulness of plant preparations in diabetes management, which will give support to the pre-clinical results.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abd el-aziz AM, Awad NE, Seida AA, El-khayat Z (2013). Biological and chemical evaluation of the use of *Acacia nilotica* (L.) in the Egyptian traditional medicine. Int. Bull. Drug. Res. 3:1-19.

Abdalrahman AAA, El Tigani S, Yagi S (2016). Biological activity of extracts from *Capparis decidua* L. twigs. J. Med. Plants Res. 10:1-7.

Abdel Motaal A, Shaker S, Haddad PS (2012). Antidiabetic activity of standardized extracts of *Balanites aegyptiaca* fruits using cell-based bioassays. Pharmacogn. J. 4:20-24.

Abdelhady MIS, Youns M (2014). *In-vitro* evaluation of the anti-diabetic activity of alcoholic extracts of certain plants belonging to families Meliaceae and Fabaceae. Nat. Prod. Indian J. 10:99-101.

Abou Khalil NS, Abou-Elhamd AS, Wasfy SIA, El Mileegy IMH, Hamed MY, Ageely HM (2016). Antidiabetic and antioxidant impacts of desert date (*Balanites aegyptiaca*) and parsley (*Petroselinum sativum*) aqueous extracts: lessons from experimental rats. J. Diabetes Res. Article ID 8408326, 10p.

Abubakar G, Danladi AA, Sunday EA, San I (2009). Antidiabetic effect of *Nauclea latifolia* leaf ethanolic extract in streptozotocin-induced diabetic rats. Pharmacogn. Res. 1:392-395.

Adam SIY, Abd Alhameed MI (2013). *Kigelia africana* fruits' extracts antihepato-toxic effects on male Wistar rats liver destruction induced by CCL_4. Asian J. Med. Sci. 5:26-32.

Afaf I, Abuelgasim A, Fatih Elruhman O, Galal M (2007). Toxicological effects of *Ambrosia maritime* L. (Compositae) extracts in rats. Vet. Res.1:71-75.

Agarwal V, Chavan BM (1988). A study on composition and hypolipidaemic effect of dietary fibre from some plant foods. Plant Foods Hum. Nutr. 38:189-197.

Agarwal V, Sharma AK, Upadhyay A, Singh G, Gupta R (2012). Hypoglycemic effects of *Citrullus colocynthis* roots. Acta Pol. Pharm. Drug Res. 69: 75.

Aguh BI, Nock IH, Ndams IS, Agunu A, Ukwubile CA (2013). Hypoglycaemic activity and nephro-protectective effect of *Bauhinia rufescens* in alloxan-induced diabetic rats. IJAPBC. 2: 249-255.

Agyare C, Dwobeng AS, Agyepong N, Boakye YD, Mensah KB, Ayande PG, Adarkwa-Yiadom M (2013). Antimicrobial, antioxidant, and wound healing properties of *Kigelia africana* (Lam.) Beneth. and *Strophanthus hispidus* DC. Adv. Pharmacol. Sci. ID 692613 10p.

Ahmad M, Zaman F, Sharif T, Ch MZ (2008). Antidiabetic and hypolipidemic effects of aqueous methanolic extract of *Acacia nilotica* pods in alloxan-induced diabetic rabbits. Scand. J. Lab. Anim. Sci. 35:29-34.

Akanni OO, Owumi SE, Adaramoye OA (2014). *In vitro* studies to assess the antioxidative, radical scavenging and arginase inhibitory potentials of extracts from *Artocarpus altilis, Ficus exasperate* and *Kigelia africana*. Asian Pac. J. Trop. Biomed. 4:492-499.

Akanya HO, Isa UL, Adeyemi HR, Ossamulu IF (2015). Effect of *Sesamum indicum* (Linn) seeds supplemented diets on blood glucose, lipid profiles and serum levels of enzymes in alloxan induced diabetic rats. J. Appl. Life Sci. Int. 2:134-144.

Akash MSH, Rehman K, Chen S (2014). Spice plant *Allium cepa*: Dietary supplement for treatment of type 2diabetes mellitus. Nutrition 30:1128-1137.

Alamin MA, Yagi AI, Yagi SM (2015). Evaluation of antidiabetic activity of plants used in Western Sudan. Asian Pac. J. Trop. Biomed. 5:395-402.

Ammar NM, Al Okbi SY, Badawy IH (1993). Hypoglycemic effect of different extracts of *Ambrosia maritima* L. Compositae. J. Islamic Acad. Sci. 6:298-301.

Amutha K, Godavari A (2016). *In-vitro* antidiabetic activity of n-butanol extract of *Sesamum indicum*. Asian J. Pharm. Clin. Res. 9:60-62.

Anitha T, Balakumar C, Ilango KB, Benedict Jose C, Vetrivel D (2014). Antidiabetic activity of the aqueous extracts of *Foeniculum vulgare* on streptozotocin-induced diabetic rats. Int. J. Adv. Pharm. Biol. Chem. 3:487-494.

Atawodi SE, Atawodi JC, PalaY, Idakwo P (2009). Assessment of polyphenol profile and antioxidant properties of leaves, stem and root barks of *Khaya senegalensis* A Juss. Electronic J. Biol. 5:80-84.

Atolani O, Adeyemi SO, Akpan E, Adeosun CB, Olatunji GA (2011). Chemical composition and antioxidant potentials of *Kigelia pinnata* root oil and extracts. EXCLI J. 10:264-273.

Auwal MS, Tijjani AN, Lawan FA, Mairiga IA, Ibrahim A, Njobdi AB, Shuaibu A, Sanda KA, Wakil AM, Thaluvwa AB (2012). The quantitative phytochemistry and hypoglycemic properties of crude mesocarp extract of *Hyphaene thebaica* (doumpalm) on normoglycemic wistar albino rats. J. Med. Sci. 12:280-285.

Auwal S, Saka S, Shuaibu A, Mairiga IA, Sanda KA, Ibrshim A, Lawan FA, Thaluvwa AB, Njobdi AB (2013). Phytochemical properties and hypoglycemic acticity of the aqueous and fractionated portions of *Acacia nilotica* (Fabaceae) pod extracts on blood glucose level in normoglycemic Wistar albino rats. J. Med. Sci. 13:111-117.

Badole SL, Ghule AE, Wagh NK (2013). Antidiabetic Activity of Allium Sativum. In Bioactive Food as Dietary Interventions for Diabetes. pp. 157-161.

Bamosa AO (2010). Effect of *Nigella sativa* seeds on the glycemic control of patients with type 2 diabetes mellitus. Indian J. Physiol. Pharmacol. 54:344-354.

Bamosa AO (2015). A review on the hypoglycemic effect of *Nigella sativa* and thymoquinone. Saudi J. Med. Med. Sci. 3:2-7.

Barapatre A, Aadil KR, Tiwary BN, Jha H (2015). *In vitro* antioxidant and antidiabetic activities of biomodified ligninfrom *Acacia nilotica* wood. Int. J. Biol. Macromolec. 75:81-89.

Batra S, Batra N, Nagori BP (2013). Preliminary phytochemical studies and evaluation of antidiabetic activity of stem bark of *Acacia senegal* (L.) Willd. in alloxan induced diabetic albino rats. Int. J. Med. Res. Rev. 1: 611-616.

Beji SR, Jameleddine S, Ksour R (2016). Assessment of the antidiabetic, antihyperlipidemic and antioxidant properties of *Trigonella foenum-graecum* Linnaeus, 1753 (Fenugreek) in alloxan-induced diabetic rats. J. New Sci. Agric. Biotechnol. 28(7):1602-1609.

Benhaddou-Andaloussi A, Martineau L, Vuong T, Meddah B, Madiraju P, Settaf A, Haddad PS (2011). The *in vivo* antidiabetic activity of *Nigella sativa* is mediated through activation of the AMPK pathway and increased muscle Glut4 content. Evid. Based Complement. Alternat. Med. Article ID 538671.

Bhardwaj N, Gauttam V, Kalia AN (2010). Evaluation of antidiabetic activity of *Momordica balsamina* Linn seeds in experimentally-induced diabetes. J. Chem. Pharm. Res. 2:701-707.

Bhuvaneswari P, Krishnakumari S (2012). Antihyperglycemic potential of *Sesamum indicum* (linn) seeds in streptozotocin induced diabetic rats. Int. J. Pharm. Pharm. Sci. 4:527-531.

Breu W, Dorsch W (1994). *Allium cepa* L. (Onion): Chemistry, analysis and pharmacology. In: Wagner H, Farnsworth N, eds. Econ. Med. Plants Res. 6:116-116.

Deferme S, Kamuhabwa A, Nshimo C, deWitte P, Augustijns P (2003). Screening of Tanzanian plant extracts for their potential inhibitory effect on *P*-glycoprote inmediatede flux. Phytother. Res. 17:459-464.

Dehghani F, Shahin MRP (2006). The toxic effect of alcoholic extract of *Citrullus colocynthis* on rat liver. Iranian J. Pharmacol. Ther. 5:117-119.

Dhriti V, Chowdary PV, Rahul J, Vishank G, Shivaji BB (2014). Free radical scavenging and anti-diabetic activity of *kigelia pinnata*. World J. Pharm. Pharm. Sci. 3:1249-1262.

Ebrahim AM, Eltayeb MH, Khalid H, Mohamed H, Abdalla W, Grill P, Michalke B (2012). Study on selected trace elements and heavy metals in some popular medicinal plants from Sudan. J. Nat. Med. 66(4):671-679.

Effiong GS, Essien GE, Ekpo AV (2014). Comparison of the antiglycemic and hypolipidaemic effects of n-hexane and methanol leaf extracts of *Nauclea latifolia* in alloxan-induced diabetic rats. Int. Res. J. Basic Clin. Stud. 2:82-86.

El Rabey HA, Al-Seeni MN, Bakhashwain AS (2017). The Antidiabetic Activity of *Nigella sativa* and Propolis on Streptozotocin-Induced Diabetes and Diabetic Nephropathy in Male Rats. Evid. Based Complement. Alternat. Med., Article ID 5439645, 14p.

Elamin A, Omer MIA, Hofvander Y, Tuvemo T (1989). Prevalence of insulin dependent diabetes mellitus (IDDM) in school children in Khartoum, Sudan. Diabetes Care. 12(6):430-432.

El-Desoky GE, Aboul-Soud MAM, Al-Numair KS (2012). Antidiabetic and hypolipidemic effects of *Ceylon cinnamon* (Cinnamomum verum) in alloxan-diabetic rats. J. Med. Plants Res. 6(9):1685-1691.

El-Ghazali GB, El Tohami MS, El Egami AB, Abdalla WS, Mohammed MG (1997). Medicinal plants of the Sudan: Part 4. Medicinal plants of Northern Kordofan. Medicinal and Aromatic Plants Research Institute. National Center for Research, Khartoum.

EL-Kamali HH, EL-amir MY (2010). Antibacterial activity and phytochemical screening of ethanolic extracts obtained from selected Sudanese medicinal plants. Curr. Res. J. Biol. Sci. 2:143-146.

El-Saadany SS, Abdel-Rahim EA, Wasif MM (1986). Biochemical action

of *Balanites aegyptiaca* fruits as a possible hypoglycemic agent. Food Chem. 19: 307-315.
El-Shora HM, El-Amier YA, Awad MH (2016). Antioxidant activity of leaf extracts from *Zygophyllum coccineum* L. collected from desert and coastal habitats of Egypt. Int. J. Curr. Microbiol. App. Sci. 5:635-641.
Ezuruike UF, Prieto JM (2014). The use of plants in the traditional management of diabetes in Nigeria: Pharmacological and toxicological considerations. J. Ethnopharmacol. 155:857-924.
Farag MA, Porzel A, Wessjohann LA (2015). Unraveling the active hypoglycemic agent trigonelline in *Balanites aegyptiaca* date fruit using metabolite fingerprinting by NMR. J. Pharm. Biomed. Anal. 115:383-387.
Festing S, Wilkinson R (2007). The ethics of animal research. EMBO Reports. 8:526-530.
François MG, Tehoua L, Ouattara H, Yapi A (2014). Comparative of the antihyperglycemic activity of Sclerocarya birrea, *Khaya senegalensis, Heliotropium indicum* and *Ocimum gratissimum* to rats wistar. Am. J. Biosci. 2:60-63.
Fröde TS, Medeiros YS (2008). Animal models to test drugs with potential antidiabetic activity. J. Ethnopharmacol. 115:173-183.
Gaber KE, Singhal U, Daowd O (2013). Hypoglycemic and hypolipidaemic effects of some common plants extract in Type 2 diabetic patients at Eldabba area (North Sudan). IOSR J. Pharm. Biol. Sci. 8:38-43.
Gidado A, Ameh DA, Atawodi SE (2005). Effect of *Nauclea latifolia* leaves aqueous extracts on blood glucose levels of normal and alloxan-induced diabetic rats. Afr. J. Biotechnol. 4:91-93.
Gidado A, Ameh DA, Atawodi SE, Ibrahim S (2008). Hypoglycaemic Activity of *Nauclea Latifolia* Sm. (Rubiaceae) in Experimental Animals. Afr. J. Tradit. Complement Altern. Med. 5: 201-208.
Greenblatt DJ, Leigh-Pemberton RA, von Moltke LL (2006). *In vitro* interactions of water-soluble garlic components with human cytochromes P450. J. Nutr. 136:806-809.
Gupta P, Bala M, Gupta S, Dua A, Dabur R, Injeti E, Mittal A (2016). Efficacy and risk profile of anti-diabetic therapies: Conventional vs traditional drugs - A mechanistic revisit to understand their mode of action. Pharmacol. Res. 113:636-667.
Gutch M, Razi SM, Kumar S, Gupta KK (2014). Diabetes mellitus: trends innorthern India. Indian. J. Endocrinol. Metab. 18:731-734.
Hafizur RM, Babiker R, Yagi S, Chishti S, Kabir N, Choudhary MI (2012). The antidiabetic effect of *Geigeria alata* is mediated by enhanced insulin secretion, modulation of β-cell function, and improvement of antioxidant activity in streptozotocin-induced diabetic rats. J. Endocrinol. 214:329-335.
Helal EGE, Abou-Aouf N, Khattab SM, Meselhy AERA, Abu-Amara TMM (2014). The Effects of *Ambrosia maritime* L. (Damsissa) on some biochemical and histological parameters of diabetic albino rats. Egyptian J. Hosp. Med. 57:612-629.
Hilmi Y, Abushama MF, Abdalgadir H, Khalid A, Khalid H (2014). A study of antioxidant activity, enzymatic inhibition and in vitro toxicity of selected traditional Sudanese plants with anti-diabetic potential. BMC Complement. Altern. Med. 14:149.
Houacine C, Elkhawad AO, Ayoub SMH (2012). A comparative study on the anti-diabetic activity of extracts of some Algerian and Sudanese plants. J. Diabetes Endocrinol. 3:25-28.
Ibrahim MA, Islam MdS (2014). Butanol fraction of *Khaya senegalensis* root modulates β-cell function and ameliorates diabetes-related biochemical parameters in a type 2 diabetes rat model. J. Ethnopharmacol. 154:832-838.
Ikram F; Hussain F (2014). Antidiabetic efficacy of *Nigella sativa* Linn. in alloxan-induced diabetic rabbits. Int. Med. J. Malaysia 13(1).
IM K, Issac A, NMJ, Ninan E, Maliakel B, Kuttan R (2014). Effects of the polyphenol content on the anti-diabetic activity of *Cinnamomum zeylanicum* extracts. Food Funct. 5(9):2208-2220.
Jayaraman R, Shivakumar A, Anitha T, Joshi VD, Palei NN (2009). Antidiabetic effect of petroleum ether. extract of *Citrullus colocynthis* fruits against streptozotocin-induced hyperglycemic rats. Rom. J. Biol. Plant Biol. 4:127-34.
Jebasingh D, Venkataraman S, Jackson DD, Emerald BS (2012). Physiochemical and toxicological studies of the medicinal plant *Cyperus rotundus* L (Cyperaceae). Int. J. Appl. Res. Nat. Prod. 5:1-8.
Jouad H, Maghrani M, Eddouks M (2002). Hypoglycemic effect of aqueous extract of *Ammi visnaga* in normal and streptozotocin-induced diabetic rats. J. Herb. Pharmacother. 2(4):19-29.
Kadan S, Saad B, Sasson Y, Zaid H (2013). *In vitro* evaluations of cytotoxicity of eight antidiabetic medicinal plants and their effect on GLUT4 translocation. Evid. Based Complement. Altern. Med. pp. 1-9.
Kamel MS1, Ohtani K, Kurokawa T, Assaf MH, el-Shanawany MA, Ali AA, Kasai R, Ishibashi S, Tanaka O (1991). Studies on *Balanites aegyptiaca* fruits, an antidiabetic Egyptian folk medicine. Chem. Pharm. Bull. (Tokyo). 39(5):1229-1233..
Khan MF, Dixit P, Jaiswal N, Tamrakar AK, Srivastava AK, Maurya R (2012). Chemical constituents of *Kigelia pinnata* twigs and their GLUT4 translocation modulatory effect in skeletal muscle cells. Fitoterapia 83:125-129.
Khlifi S, Ben Jemaa H, Ben Hmad H, Abaza H, Karmous I, Abid A, Benzarti A, Elati J, Aouidet A (2016). Antioxidant, antidiabetic and antihyperlipidemic effects of *Trigonella foenum-graecum* seeds. Int. J. Pharmacol. 12:394-400.
Kiendrebeogo M, Dijoux-Franca M, Lamien CE, Meda A, Wouessidjewe D, Nacoulma OG (2005). Acute toxicity and antioxydant property of *Striga hermonthica* (Del.) Benth (Scrophulariaceae). Afr. J. Biotechnol. 4:919-922.
Kumar S, Kumar V, Prakash OM (2012). Antidiabetic and hypolipidemic activities of *Kigelia pinnata* flowers extract in streptozotocin induced diabetic rats. Asian Pac. J. Trop. Biomed. 2:543-546.
Mali RG, Mahale NB (2008). Evaluation of *Rhynchosia minima* (Linn.) DC leaves for anthelmintic activity. Int. J. Pharm. Sci. Nanotechnol. 1(2):191-194.
Mansour HA, Newairy AA, Yousef MI, Sheweita SA (2002). Biochemical study on the effects of some Egyptian herbs in alloxan-induced diabetic rats. Toxicology 170(3):221-228.
Mariod AA, Abdelwahab SI (2012). *Sclerocarya birrea* (Marula), An African tree of nutritional and medicinal uses: A review. Food Rev. Int. 28:375-388.
Mariod AA, Abdelwahab SI, Elkheir S, Ahmed JM, Fauzi PNM, Chuen ChS (2012). Antioxidant activity of different parts from *Annona squamosa*, and *Catunaregam nilotica* methanolic extract. Acta. Sci. Pol. Technol. Aliment. 11: 249-257.
Markowitz JS, DeVane CL, Chavin KD, Taylor RM, Ruan Y, Donovan JL (2003). Effects of garlic (*Allium sativum* L.) supplementation on cytochrome P450 2D6 and 3A4 activity in healthy volunteers. Clin. Pharmacol. Ther. 74(2):170-177.
Marles RJ, Farnsworth NR (1995). Antidiabetic plants and their active constituents. Phytomedicine 2:137-189.
Mikaili P, Maadirad S, Moloudizargari M, Aghajanshakeri S, and Sarahroodi S (2013). Therapeutic uses and pharmacological properties of garlic, shallot, and their biologically active compounds. Iran J. Basic. Med. Sci. 16:1031-1048.
Mogale MA, Lebelo SL, Thovhogi N, de Freitas AN, Shai LJ (2011). α-Amylase and α-glucosidase inhibitory effects of *Sclerocarya birrea* (A. Rich.) Hochst. subspecies *caffra* (Sond) Kokwaro (Anacardiaceae) stem-bark extracts. Afr. J. Biotechnol. 10:15033-15039.
Moller DE (2001). New drug targets for type 2 diabetes and the metabolic syndrome. Nature. 414:821-827.
Motala AA, Omar MA, Pirie FJ (2003). Diabetes in Africa. Epidemiology of type 1 and type 2 diabetes in Africa. J. Cardiovasc. Risk. 10(2):77-83.
Mousinho NMH, van Tonder JJ, Steenkamp V (2013). *In vitro* anti-diabetic activity of *Sclerocarya birrea* and *Ziziphus mucronata*. Nat. Prod. Commun. 8:1279-84.
Muhammad A, Sira HM (2013). Antimicrobial, antityrosinase and brine shrimp lethality test of *Bauhinia rufescens* Lam (Fabaceae). J. Coast Life Med. 1(2):135-140.
Musa MS, Abdelrasoo FE, Elsheikh EA, Ahmed LAMN, Mahmoud AE, Yagi SM (2011). Ethnobotanical study of medicinal plants in the Blue Nile State, south-eastern Sudan. J. Med. Plants Res. 5: 4287-4297.
Mustafa AH, Eltayeb BI, Ali MA, Shaddad AS, Mohammad HA (2013). Antidiabetic and hypolipidaemic effects of *Cicer arientinum* seedsextracts in hyperglycemic and diabetic rats. J. Pharm. Biomed. Sci. 30:1046-1052.
Mustafa HA, Eltaeb IB, Ali MA, Shadad SA (2010). Antidiabetic and hypolipidaemic effects of *Cinnanomum verum* bark on

hyperglycaemic and diabetic rats. Res. J. Pharmacol. 4:21-25.
Nakhaee A, Bokaeian M, Saravani M, Farhangi A, Akbar-zadeh A (2009). Attenuation of oxidative stress in streptozotocin-induced diabetic rats by *Eucalyptus globulus*. Indian J. Clin. Biochem. 24(4):419-425.
Ojewole JAO (2003). Hypoglycemic effect of *Sclerocarya birrea* (A. Rich.) Hochst. (Anacardiaceae) stem-bark aqueous extract in rats. Phytomedicine 10:675-681.
Okoro SO, Kawo AH, Arzai AH (2012). Phytochemical screening, antibacterial and toxicological activities of *Acacia senegal* extracts. Bayero J. Pure Appl. Sci. 5(1):163-170.
Oluwakanyinsola SA, Adeniyi TY, Akingbasote JA, Florence OE (2010). Acute and subacute toxicity study of ethanolic extract of the stem bark of *Faidherbia albida* (DEL) A. chev (Mimosoidae) in rats. Afr. J. Biotechnol. 9:1218-1224.
Oryan A, Hashemnia M, Hamidi A, Mohammadalipour A (2014). Effects of hydro-ethanol extract of *Citrullus colocynthis* on blood glucose levels and pathology of organs in alloxan-induced diabetic rats. Asian Pac. J. Trop. Dis. 4(2):125-130.
Ozougwu J, Eyo J (2010). Studies on the anti-diabetic activity of *Allium sativum* (garlic) aqueous extracts on alloxan-induced diabetic albino rat. Pharmacologyonline 2:1079-1088.
Pal R, Hooda MS, Bias CS, Singh J (2014). Hepatoprotective activity of *Acacia senegal* pod against carbon tetrachloride-induced hepatotoxicity in rats. Int. J. Pharm. Sci. 26:165-168.
Patra A, Jha S (2009). Antidiabetic effect of the aqueous extract of *E. citriodora* in alloxan induced diabetic rats. Pharmacog. Mag. 5:51-54.
Priya B, Gahlot M, Joshi P (2014). Screening of anti-hyperglycemic activity of *Kigelia africana* on alloxan-induced diabetic rats. Indian J. Appl. Res. 4:448-451.
Rahma EH, Narasinga RMS (1984). Effect of debittering treatment on the composition and protein components of lupin seeds (*Lupinus termis*). J. Agric. Food Chem. 32(5):1026-1030.
Rotenstein LS, Kozak BM, Shivers JP, Yarchoan M, Close J, Close KL (2012). The ideal diabetes therapy: what will it look like? How close are we?. Clin. Diabetes 30:44-53.
Salib JY, Michael HN, Eskande EF (2013). Anti-diabetic properties of flavonoid compounds isolated from *Hyphaene thebaica* epicarp on alloxan induced diabetic rats. Pharmacogn. Res. 5:22-29.
Sathiavelu A, Sangeetha S, Archit R, Mythili S (2013). *In vitro* anti-diabetic activity of aqueous extract of the medicinal plants *Nigella sativa, Eugenia jambolana, Andrographis paniculata* and *Gymnema sylvestre*. Int. J. Drug Dev. Res. 5(2):323-328.
Sewani-Rusike CR, Jumbam DN, Chinhoyi LR, Nkeh-Chungag BN (2015). Investigation of hypogycemic and hypolipidemic effects of an aqueous extract of *Lupinus albus* legume seed in streptozotocin induced type I diabetic rats. Afr. J. Tradit. Complement. Altern. Med. 12:36-42.
Sharma RD, Sarkar A, Hazar DK, Misra B, Singh JB, Maheshwari BB (1996). Toxicological evaluation of fenugreek seeds: a long term feeding experiment in diabetic patients. Phytother. Res. 10: 519-520.
Shaw D, Graeme L, Pierre D, Elizabeth W, Kelvin C (2012). Pharmacovigilance of herbal medicine. J. Ethnopharmacol. 140:513-518.
Shaw JE, Sicree RA, Zimmet PZ (2010). Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes. Res. Clin. Pract. 87:4-14.
Sheehan MT (2003). Current therapeutic options in type 2 diabetes mellitus: a practical approach. J. Clin. Med. Res. 1:189-200.
Sheweita SA, Newairy AA, Mansour HA, Yousef MI (2002). Effect of some hypoglycemic herbs on the activity of phase I and II drug-metabolizing enzymes in alloxan-induced diabetic rats. Toxicology 174(2):131-139.
Sikder MAA, Hossian AN, Parvez MM, Kaisar MA, Nimmi I, Rashid MA (2011). Antioxidant behavior of two Bangladeshi medicinal plants: *Kigelia pinnata* and *Mesua nagassarium*. Bangladesh Pharm. J. 14:27-30.
Simeonova R, Burdina MK, Dimitrova DZ, Gevrenova R, Balabanova V, Vitcheva V, Tzankova V, Kasabova A (2016). Antidiabetic, antioxidant and antihypertensive effects of 3,5-dicaffeoylquinic acid from *Geigeria alata* (Dc) Oliv. & Hiern. on streptozotocin-induced diabetic normotensive and hypertensive rats.Toxicol. Lett. 258:S293.
Singh P, Khosa RL, Mishra G, Jha KK (2015). Antidiabetic activity of ethanolic extract of *Cyperus rotundus* rhizomes in streptozotocin-induced diabetic mice. J. Pharm. Bioallied. Sci. 7:289-292.
Sylvester EG, Dan AH (2015). The effect of *Nauclea latifolia* leaf extract on some biochemical parameters in streptozotocin diabetic rat models. J. Med. Med. Sci. 6:47-52.
Taj Eldin IM, Ahmed EM, Mohamedm AH (2009). Clinical hypoglycemic effects of *Allium cepa* (red onion) in type 1 diabetic patients. Sudan J. Med. Sci. 4:153-157.
Takeuchi H, Mooi LY, Inagaki Y, He P (2001). Hypoglycemic effect of a hot-water extract from defatted sesame (*Sesamum indicum* L.) seed on the blood glucose level in genetically diabetic KK-Ay mice. Biosci. Biotechnol. Biochem. 65:2318-2321.
Tanko Y, Muhammad A, Elaigwu J, Mohammed KA, Jimoh A, Sada NM, Dallatu, Yerima M, Mohammed A (2013). Anti-diabetic effects of ethyl acetate and n-butanol fractions of *Acacia nilotica* leaves methanolic extract on alloxan-induced diabetic Wistar rats. J. Appl. Pharm. Sci. 3:089-093.
Thompson PL, Davis TME (2017). Cardiovascular effects of glucose-lowering therapies for type 2 diabetes: New drugs in perspective. Clin. Ther. 39(5):1012-1025.
Thomson M, Al-Amin ZM, Al-Qattan KK, Shaban LH, Ali M (2007). Anti-diabetic and hypolipidaemic properties of garlic (*Allium sativum*) in streptozotocin-induced diabetic rats. Int. J. Diabetes & Metabolism 15: 108-115.
Thomson M, Al-Qattan KK, Divya JS and Ali M (2016). Anti-diabetic and anti-oxidant potential of aged garlic extract (AGE) in streptozotocin-induced diabetic rats. BMC Complement. Alternat. Med. 16:17.
Traore F, Gasquet M, Laget M, Guiraud H, Di Giorgio C, Azas N, Doumbo O, Timon-David P (2000). Toxicity and genotoxicity of antimalarial alkaloid rich extracts derived from *Mitragyna inermis* O. Kuntze and *Nauclea latifolia*. Phytother. Res. 14:608-611.
Udayasekhara RP, Sesikeran B, Srinivasa RP (1996). Short term nutritional and safety evaluation of fenugreek. Nutr. Res. 16:1495-1505.
Umar IA, Mohammad A, Ndidi US, Abdulazeez AB, Olica WC, Adam M (2014). Antihyperglycemic and antihyperlipidemic effect of aqueous stem bark extract of *Acacia albida* Delile in alloxan-induced diabetic rats. Asian J. Biochem. 9:170-178.
Vadnere GP, Patil AV, Wagh SS, Jain SK (2012). In vitro free radical scavenging and antioxidant activity of *Cicer arietinum* L. (Fabaceae). Int. J. Pharm Tech. Res. 4(1):343-350.
van de Venter M, Roux S, Bungu LC, Louw J, Crouch NR, Grace OM, Maharaj V, Pillay P, Sewnarian P, Bhagwandin N, Folb P (2008). Antidiabetic screening and scoring of 11 plants traditionally used in South Africa. J. Ethnopharmacol. 119:81-86.
Wichitsranoi J, Weerapreeyakul N, Boonsiri P, Settasatian C, Settasatian N, Komanasin N, Sirijaichingkul S, Teerajetgul Y, Rangkadilok N, Leelayuwat N (2011). Antihypertensive and antioxidant effects of dietary black sesame meal in pre-hypertensive humans. Nutr. J. 10:82.
Yadav BV, Deshmukh TA, Badole SL, Kadam HM, Bodhankar SL, Dhaneshwar SR (2009). Antihyperglycemic activity of *Cicer arietinum* seeds. Pharmacologyonline 3:748-757.
Yadav P, Sarkar S, Bhatnagar D (1997). Action of *Capparis deciduas* against alloxan-induced oxidative stress and diabetes in rat tissues. Pharmacol. Res. 36:221-228.
Yagi S, Abd Rahman AE, ELhassan GOM, Mohammed AMA (2013). Elemental analysis of ten Sudanese medicinal plants using X -ray fluorescence. J. Appl. Ind. Sci. 1:49-52.
Yagi SM, El Tigani S, Adam SEI (1998). Toxicity of *Senna obtusifolia* fresh and fermented leaves (Kawal), *Senna alata* leaves and some products from *Senna alata* on rats. Phytother. Res. 12:324-330.
Yang CH, Li RX, Chuang LY (2012). Antioxidant activity of various parts of *Cinnamomum cassia* extracted with different extraction methods. Molecules, 17(6):7294-7304.
Zaid H, Rayan A, Said O, Saad B (2010). Cancer treatment by Greco-Arab and Islamic herbal medicine. Open Nutraceuticals J. 3:203-212.
Zia-Ul-Haq M, Ćavar S, Qayum M, Imran I, de Feo V (2011). Compositional studies: antioxidant and antidiabetic activities of *Capparis decidua* (Forsk.) Edgew. Int. J. Mol. Sci. 12:8846-8861.

academicJournals

Vol. 12(3), pp. 41-51, 22 January, 2018
DOI: 10.5897/AJPP2017.4860
Article Number: 7B7C12355849
ISSN 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Evaluation of antihypertensive and vasorelaxant effects of *Heimia salicifolia* (family: Lythraceae)

Elizabeth A. Guzmán-Hernández[1], David Segura-Cobos[1], Dante Amato[1], José Guillermo Ávila-Acevedo[2] and Beatriz Vázquez-Cruz[1]*

[1]Laboratorio de Farmacología, Unidad de Investigación Interdisciplinaria en Ciencias de la Salud y la Educación (UIICSE), Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México (UNAM), Tlalnepantla, México.
[2]Laboratorio de Fitoquímica, Unidad de Biología, Tecnología y Prototipos (UBIPRO), Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México (UNAM), Tlalnepantla, México.

Received 18 October, 2017; Accepted 10 January, 2018

There are reports of the presence of vasodilatory alkaloids in *Heimia salicifolia* extracts. The action mechanisms of the potential antihypertensive effect of such alkaloids have not however, been elucidated. The objective of the present study was to corroborate the antihypertensive and vasorelaxant activities of the isolated alkaloids from *H. salicifolia*, and to characterize their vasorelaxant actions. A chloroform extract (HSCE) was obtained from *H. salicifolia.* The alkaloids were separated by thin layer chromatography, extracted from silica gel, and evaluated using spectroscopy. The alkaloid with the most potent vasorelaxant activity was identified as lythrine. The antihypertensive effect of the HSCE was corroborated on normotensive rats and on animals with N^{ω}-nitro-L-arginine-methyl ester (L-NAME) induced hypertension. The action mechanisms of the HSCE and lythrine were studied on isolated and perfused rat mesenteric vascular bed (MVB) preparations. HSCE administration produced a concentration-dependent relaxation, but in preparations without vascular endothelium, the relaxant response to HSCE was significantly diminished. In MVB preparations with intact vascular endothelium, pre-contracted with phenylephrine or L-NAME, perfusion with lythrine produced concentration-dependent relaxation; a similar effect was produced by acetylcholine. Wortmannin (phosphatidylinositol 3-kinase inhibitor) and methylene blue (guanylate cyclase inhibitor) decreased the relaxant effect of lythrine. These data suggest that the vascular relaxation induced by alkaloids isolated from *H. salicifolia* is dependent on the activation of the nitric oxide/guanylate cyclase pathway.

Key words: *Heimia salicifolia,* lythrine alkaloid, hypertension, N^{ω}-nitro-L-arginine-methyl ester (L-NAME), mesenteric vascular bed.

INTRODUCTION

Hypertension can be defined as chronic elevation of systolic and/or diastolic blood pressure above 140/90 mm

*Corresponding author. E-mail: bvcruz@unam.mx. Tel: +52 55 5623-1333 Ext. 39768, 39797. Fax: +52 55 5390-7604.

Hg. It is a major risk factor for cardiovascular diseases such as congestive heart failure, coronary artery disease, stroke, and renal disease. Despite progress in prevention, detection, treatment, and control of high blood pressure, hypertension remains an important public health problem (Go et al., 2014), and its prevalence has been gradually increasing worldwide. The World Health Organization (WHO) reported in 2013 that hypertension is a major public health issue and it is the cause one in every eight deaths. Globally, cardiovascular diseases account for approximately 17 million deaths/year, and complications of hypertension account for 9.4 million deaths/year worldwide (World Health Organization, 2013). In Mexico, hypertension is considered a national health problem (Balam-Ortiz et al., 2014). Hypertension prevalence in Mexican adults ≥ 20 years was 23.8% in 1993, 30.8% in 2006, and 31.5% in 2012 (Campos-Nonato et al., 2013). Many synthetic antihypertensive drugs have been commonly used for hypertension control in developed countries. Some of these drugs have many side effects. Complementary and alternative medicine may have the potential for hypertension treatment (Magos et al., 2017; Baharvand et al., 2017). Herbal medicines still remain the most popular choice in certain developing countries. The extensive use of herbal remedies in such nations has led to extensive research in this area to determine their potential efficacy. Several modern cardiovascular drugs are now available as natural/herbal products (Tirapelli et al., 2010; Luna et al., 2013).

There are several regions in Mexico with many plants used as herbal remedies that may represent an opportunity for the discovery of new antihypertensive drugs (Hernández et al., 2013). Also, it has been demonstrated that a partial separation of *H. salicifolia* alkaloids produced antihypertensive effects on angiotensin II-caused acute hypertension. Nevertheless, the pharmacological properties of *H. salicifolia* have not yet been widely studied. The aim of the present study was to investigate the antihypertensive and vasorelaxant activity of the isolated alkaloids from *H. salicifolia* and to characterize its possible vasorelaxant action.

MATERIALS AND METHODS

Study species

Heimia salicifolia (H.B&K) Link & Otto (Lythraceae family) is a wild flowering shrub distributed over Mexico, Western Texas, El Salvador, Jamaica, Uruguay, and Argentina (Malone and Rother, 1994). This plant is known as sun opener (Graham, 1997). In Mexico, the most common name is sinicuichi (Guzman et al., 2006). *H. salicifolia* has been used in folk medicine in several countries for different indications, as an emetic, haemostatic, tonic laxative, diuretic, anti-inflammatory, and to treat some syphilis symptoms (Malone and Rother, 1994; Aguilar et al., 1994). Due to its psychotomimetic activity, native people in Central America and Mexico have used the plant for shamanic purposes (Graham, 1997). After the administration of alcohol decoction or plant juice, the subjects experiment a variety of effects such as yellow-colored vision and auditory distortion in which bells or voices sound as if they were originated in farther distances (Malone and Rother, 1994). Phytochemical analyses have shown that *H. salicifolia* contain biphenylquinolizidine lactone alkaloids (Malone and Rother, 1994). More than 20 of these alkaloids such as vertine, sinicuichine, lythrine, nesodine, lyfoline, dehydrocodeine, and demethyllasubine I, have been isolated (Blomster et al., 1964; Malone and Rother, 1994). Vertine, lyfoline, lythrine, and nesodine seem to be the primary source of the traditional effects of *H. salicifolia*. It also has been reported that these alkaloids possess cardiovascular effects; nesodine produced a fall in blood pressure (Malone and Rother, 1994; Kaplan and Malone, 1966). Another reported effect for cryogenine and nesodine is the inhibition of prostaglandin synthase, which may support the folklore anti-inflammatory usage of *H. salicifolia*. Cryogenine has been reported to have anti-inflammatory activity similar to that of aspirin (Lema et al., 1986). Rumalla et al. (2008) isolated two more alkaloids from *H. salicifolia* and elucidated its chemical structure using spectroscopic techniques. The new alkaloids showed antimalarial activity. There are no ethnopharmacological reports of *H. salicifolia* being used as an antihypertensive drug. It has been found that the aqueous extract from *H. salicifolia* leaves decreased the systolic blood pressure in anaesthetized normotensive rats.

Chemicals and drugs

Phenylephrine bitartrate (Phen), acetylcholine chloride, N-nitro-L-arginine methyl ester (L-NAME), atropine, methylene blue, wortmannin and diphenhydramine were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). All other reagents were analytical grade from Merck, Germany.

Experimental animals

The experiments were performed in male Wistar rats (300-350 g) breed in the Animal Facility of the Facultad de Estudios Superiores Iztacala (FESI), Universidad Nacional Autónoma de México (UNAM). Female animals were not studied to avoid physiological changes due to oestrus cycle. Animals were housed under conditions of controlled temperature (24±0.5ºC) and with 12 h light/12 h dark photoperiod. They were fed rat chow (Ralston-Purina) and water *ad libitum.* All animal procedures and protocols were conducted in accordance with the Federal Regulations for Animal Experimentation and Care (NOM-062-ZOO-1999, Ministry of Agriculture, Mexico) on care and use of laboratory animals, and approved by the institutional ethics review board.

Plant material

Dried leaves of *H. salicifolia* were obtained from the Sonora Market, a medical plants selling place in Mexico City. The plant's botanical identity was verified at the Izta Herbarium of the Botanic Department, Facultad de Estudios Superiores Iztacala and a voucher specimen numbered 41653 was deposited at the same herbarium for reference.

Preparation of chloroform extract and alkaloids isolation

A published method for chloroform extract preparation and alkaloids isolation from *H. salicifolia* was used (Blomster et al., 1964). Briefly, 5 kg of *H. salicifolia* leaves were dried at 45ºC and powdered. Afterwards, to remove fat, 4 L of petroleum ether (J.T. Baker) was added; then the plant material was macerated in 5 L of methanol

(J.T. Baker) during 24 h, and concentrated in a rotary evaporator (Buchi Rotavapor model Mp60) under reduced pressure to get a final volume of 50 mL. Alkaloids detection was carried out with Dragendorff reagent. Methanol concentrate was dried at 50ºC for 24 h, in addition to 100 mL distilled water and acidified to pH 2.0 with 10% hydrochloric acid solution (Tec. Chem.), and filtered through celite (Sigma Chem.); the precipitate was washed with distilled water. The aqueous acidic filtrate was further defatted in a continuous extractor with 500 mL ethyl ether. The pH was adjusted to 9 with 28% ammonium hydroxide solution, continuously extracted with 200 mL chloroform. This *H. salicifolia* chloroform extract (HSCE) was dried *in vacuum* at 40ºC with a yield of 15 g (approximately 0.3%). HSCE was used for biological experiments and for alkaloids isolation.

Alkaloids isolation from HSCE

Dried HSCE was dissolved in chloroform, adsorbed on basic alumina (J.T. Baker), dried, and placed on the top of a column (70 × 2 cm) of basic alumina (J.T. Baker). Elution was done with a mixture of methanol/chloroform (1:1) and finally with methanol. The effluent was collected in three fractions. Each fraction was dried *in vacuum* and chemical tests were done for alkaloids. Thin-layer preparative chromatography of the fractions was performed on silica gel G plates (Merck), with chloroform/methanol (3:2) (v/v); the chromatograms were observed with an ultraviolet lamp (UVGL-25) at 250 nm. The separated fractions were marked, eluted from the plate and examined again for alkaloids with 4-nitroanilindiazothized reagent; thereafter, four principal alkaloids were separated and assayed in biological tests. The alkaloid with higher biological activity was the alkaloid 3 (Guzman et al., 2006), which was isolated and prepared for chemical identification by NMR studies.

Alkaloid identification

^{1}H-NMR spectrum was recorded at 500 MHz ^{13}C-NMR at 75 MHz, on a Varian Inova; chemical shifts (PPM) were relative to $(CH_3)_4$ Si as internal reference, and $CDCl_3$ (Aldrich) as solvent. The ^{1}H NMR and ^{13}C NMR data were obtained on a Varian Unity 300 instrument. Comparisons were made with spectra known of these alkaloids using the Organic Chemistry Data Base program.

Effect of HSCE on blood pressure in normotensive rats

The rats were randomly allocated into groups of six each. The Group 1 (control) received vehicle (carboxymethyl cellulose), whereas Groups 2, 3, and 4 received 10, 20, and 40 mg/kg of HSCE for 10 days p.o., respectively. At the beginning and end of treatment, systolic arterial blood pressure (SBP) was measured non-invasively using a tail-cuff computer-aided monitoring device (Automatic Blood Pressure Computer, Model LE 5007; Letica Scientific Instruments, Barcelona, Spain) as follows. The rat was restrained in a size-matched plastic container, and an inflatable latex ring containing a sensor was placed over a tail artery while the rat was kept warm (37°C) in the same device. The rats were trained to be inside the container with the cuff placed on the tail and to get used to the inflation and deflation of the latex ring. The SBP measurements were recorded for each group, and the mean of three measurements obtained in one session was considered.

Effect of HSCE on blood pressure during chronic hypertension caused by inhibition of nitric oxide synthase

Rats were randomly allocated into groups of six each. Group 1 (control) received vehicle; Group 2- N^{ω}-nitro-L-arginine-methyl ester (L-NAME) (70 mg/Kg, Sigma-Aldrich, St. Louis, MO, USA); Group 3- L-NAME + enalapril (5 mg/kg/day, Sigma-Aldrich, St. Louis, MO, USA); Group 4- L-NAME + HSCE (10 mg/kg/day); and Group 5- L-NAME + HSCE (20 mg/kg/day) for 10 days. All drugs were administered in carboxymethyl cellulose and L-NAME in the drinking water. The actual doses in each group were calculated from the daily water intake. SBP measurement was done using the aforementioned technique.

Rat mesenteric vascular bed assay: functional and mechanistic approaches

The experimental assays were performed in normotensive rat mesenteric vascular bed (MVB) isolated and perfused as a model of vascular resistance (Sousa et al., 2017). Briefly, rats were anaesthetized with sodium pentobarbital (35 mg/kg, i.p). The abdominal cavity was opened and the intestinal loops exposed; the superior mesenteric artery was dissected near to its origin in the abdominal aorta and cannulated with a PE-50 polyethylene (BD Intramedic, Oxford, U.K.) catheter. The vascular bed was covered with moist gauze and perfused with Krebs' solution, with the following mM composition: 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 NaH_2PO_4, 4.2 $MgSO_4$, 25 $NaHCO_3$, and 11.5 glucose. The solution was bubbled with O_2/CO_2 (95:5) mixture, at 37°C with a 7.4 final pH. The intestinal loops were removed as a block and the MVB was separated cutting near to the intestinal loops. The MVB was placed in a chamber at 37°C and the cannulated superior mesenteric artery connected to a peristaltic pump (Vera, Manostat, Division of Barnant Company) to provide a constant flow, adjusted to obtain a 48-50 mm Hg basal perfusion pressure. Mean flow rate was 4±0.2 $ml.min^{-1}$ whereas perfusion pressure was measured using a pressure transducer (Mod. P1000-A, Narco Bio Systems Inc., Houston Texas) placed in the circuit between the outlet of the pump and the preparation, and recorded on a Narco physiograph (Model DMP-4B, Narco Bio Systems Houston Texas). The flow was maintained at a constant rate, so vasoconstriction was recorded as an increase in perfusion pressure, and relaxation as a decrease in perfusion pressure. Data are expressed as changes (Δ) of the perfusion pressure in mm Hg. All preparations were allowed to equilibrate for at least 30 min before starting the experiments.

Phenylephrine-induced vasoconstriction

To measure the MVB vasoconstrictor response, concentration-response curves to phenylephrine (Phen) were constructed. Increasing Phen doses (0.5 to 80 µg) were injected in bolus with a 50 µL Hamilton syringe. The injection volume was 0.1 mL. The interval between injections was 5 min or the time needed for the perfusion pressure to return to its initial value, which was never longer than 10 min. In other preparation, the MVB was constricted with Krebs' solution containing Phen (10^{-5} M) to induce submaximal vasoconstriction (about 90%) and allowed to reach a plateau. When the MVB contraction was stable (30-45 min), concentration-response curves to acetylcholine (ACh) were performed; ACh was injected in 0.1 mL volumes and 10-90 µg doses, causing a dose-dependent relaxation, recorded as a decrease in perfusion pressure. The injection duration time was 10 s. Concentration-response curves to HSCE (10-90 µg) were drawn using the same method. In some preparations, the endothelium was removed by perfusion with distilled water for 10 min (Criscione et al., 1984). Concentration-response curves were constructed using the previously described parameters with the MVB pre-contracted with Phen. A bolus injection of ACh (80 µg) was applied. Vascular endothelium removal was confirmed by the absence of a relaxation response. To study the possible vasorelaxant mechanism exerted

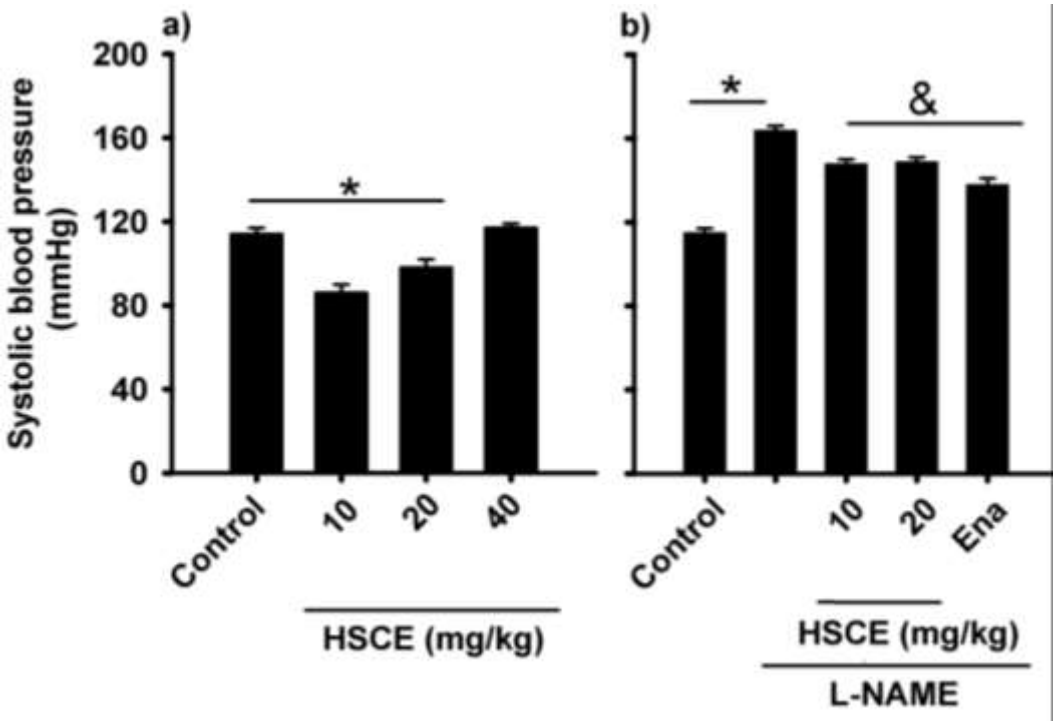

Figure 1. Effect of treatment with HSCE (10, 20 and 40 mg/kg) and enalapril (Ena 5 mg/kg) for 10 days on SBP. a) normotensive rats; b) N^{ω} -nitro-L-arginine-methyl ester (L-NAME) treated rats. Values represent the means ± SEM of 6 rats per group. *p< 0.05 vs control; $^{\&}$p< 0.05 vs L-NAME.

by the alkaloid 3 isolated from HSCE, MVB with intact endothelium was perfused for 10 min with L-NAME (10^{-4} M), (unspecific nitric oxide synthase inhibitor), methylene blue (10^{-5} M) (soluble guanylate cyclase inhibitor), wortmannin (10^{-5} M) (phosphatidylinositol 3-kinase inhibitor). The MVB was perfused for 10 min with atropine (10^{-5} M) (muscarinic cholinergic receptors antagonist), and/or diphenhydramine (10^{-5} M) (histamine H_1 receptor antagonist) and concentration-response curves to alkaloid 3 (20-80 µg in bolus) were performed. A maximum of 3 curves per preparation was constructed.

Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM) of at least five experiments. Means were compared by unpaired Student's *t*-test; concentration-response curves by two-ways ANOVA and Bonferroni's post-hoc test. Differences were considered to be statistically significant with $p \leq 0.05$.

RESULTS

HSCE effects on SBP of normotensive and hypertensive rats

Oral HSCE administration of 10 and 20 mg/kg for 10 days decreased the SBP in normotensive rats (from 115±3 to 85.5±4.5, and 96.8±4.2 mm Hg respectively); the 40 mg/kg dose did not cause SBP reduction (Figure 1a). Chronic NO inhibition by L-NAME was associated with a progressive rise in the SBP, reaching a maximum of 164±2 mm Hg at Day 10, as compared with the control group (115±3 mm Hg). HSCE administration (10 and 20 mg/kg, v. o) produced SBP reduction (148±3 mm Hg). Enalapril also reduced SBP to 131±2.5 mm Hg (Figure 1b).

Effect of HSCE on MVB from normotensive rats with and without endothelium

Baseline perfusion pressure of isolated and perfused MVB from normotensive rats was 50±2.2 mm Hg. Phen (0.5-80 µg) bolus administration induced a concentration-dependent vasoconstriction; 40 µg of Phen increased the perfusion pressure to 84±1.2 mm Hg. Constant perfusion with Phen (10^{-5} M) added to the Krebs' solution increased the perfusion pressure to 84±1.2 mm Hg. When the perfusion pressure reached a plateau (Figure 2), an ACh bolus injection produced concentration-dependent relaxation, with 90 µg of ACh reducing the perfusion pressure to 25±1 mm Hg (Figure 3a). HSCE administration (10-90 µg) produced concentration-dependent relaxation whereas 70 µg of HSCE decreased the perfusion pressure to 27±3 mm Hg (Figure 3b). When vascular endothelium was removed and pre-contracted with Phen, the relaxant response to ACh and HSCE was significantly diminished (Figures 3a and 3b).

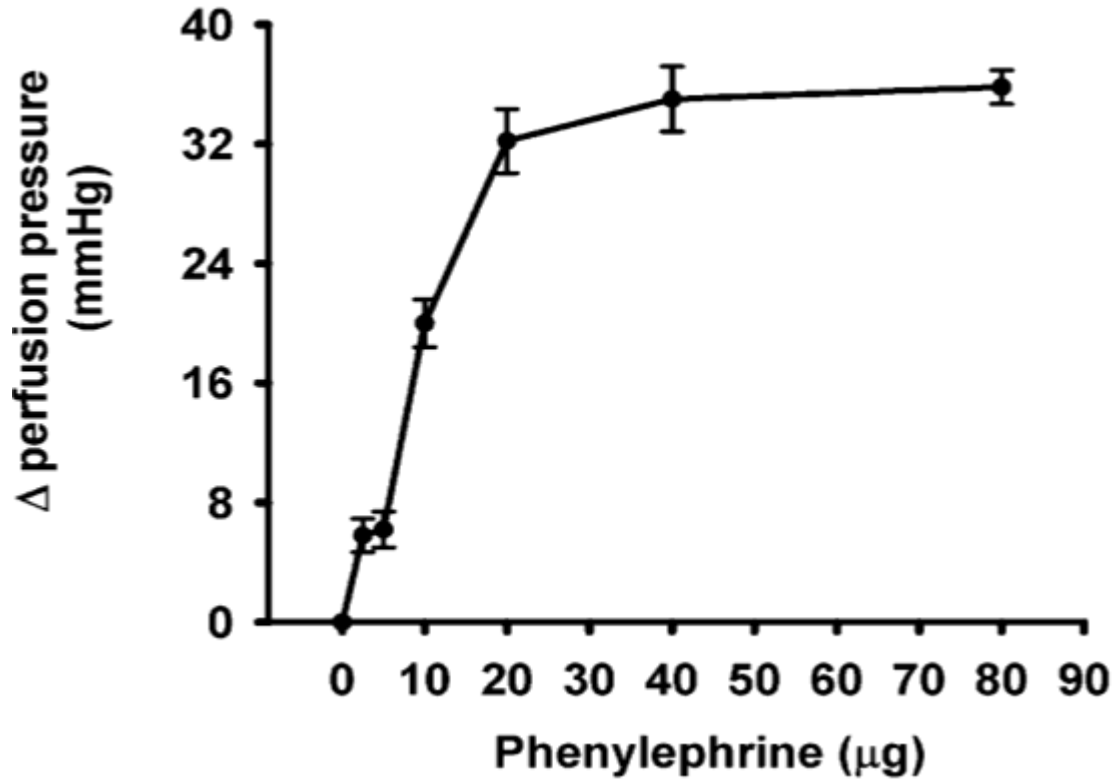

Figure 2. Phenylephrine-dependent vasoconstriction of isolated perfused mesenteric vascular bed of rats, with intact endothelium. Each point represents the mean ± S.E.M., from 6 experiments. *p< 0.05.

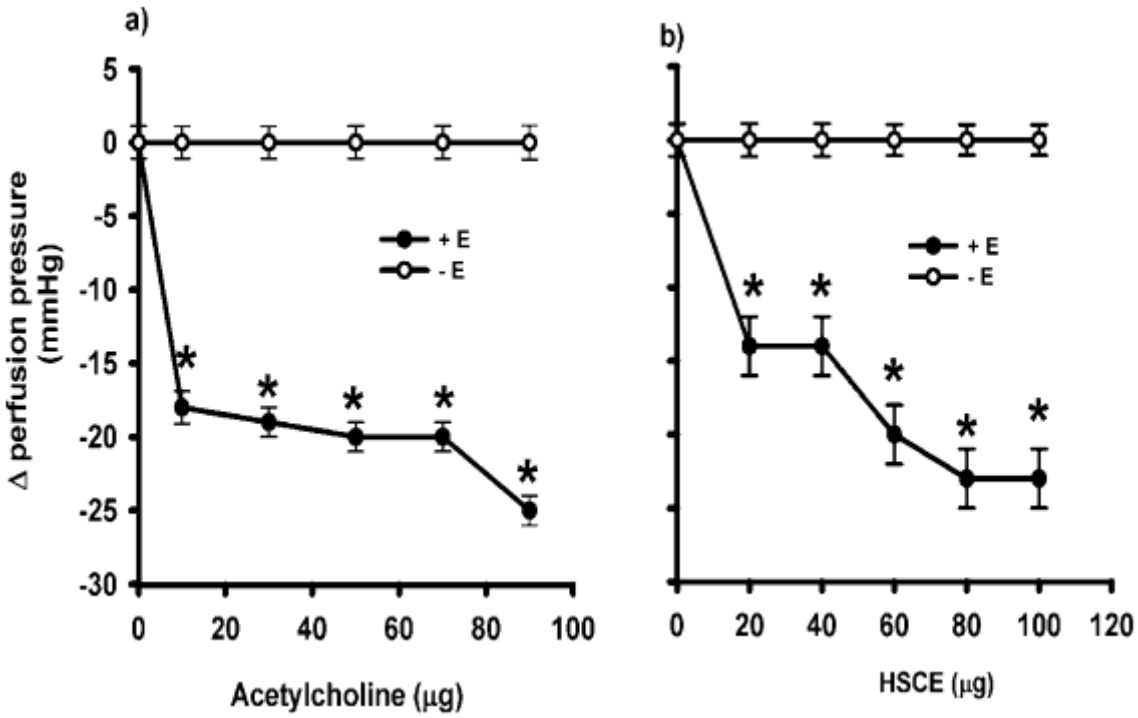

Figure 3. Effect of acetylcholine (a) and HSCE (b) on the relaxation of isolated perfused mesenteric vascular bed of rat phenylephrine (10^{-5} M) pre-contracted in the presence (+E) or absence (−E) of functional endothelium. Each point represents the mean ± S.E.M., from 6 experiments. *p< 0.05.

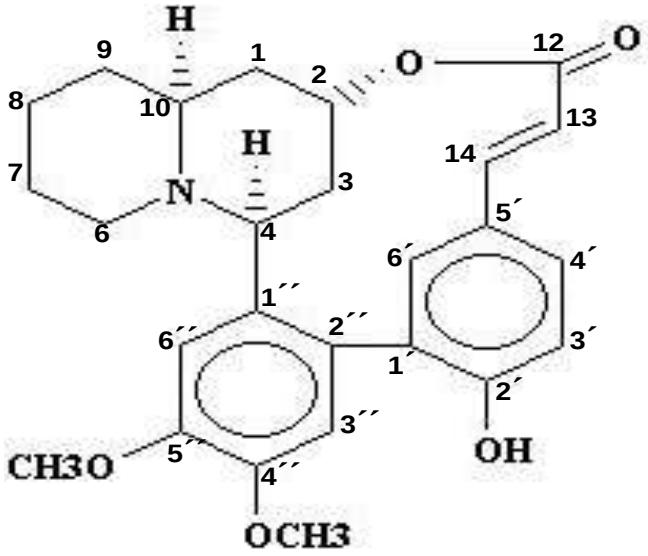

Figure 4. Chemical structure obtained with ^{13}C NMR and spectrum ^{1}H NMR from purification of alkaloid 3 obtained from *H. salicifolia* chloroform extract (HSCE). This chemical structure corresponds to lythrine.

Table 1. ^{1}H and ^{13}C NMR chemical shift assignments of alkaloid 3 (lythrine) in $CDCl_3$.

Position	^{1}H	*J*(Hz)	^{13}C	Position	^{1}H	*J*(Hz)	^{13}C
1	1.69-1.75		37.05	2'			154.09
2	5.43*br, s*		70.98	3'	6.90*d*	8.2	116.17
3	2.42*d* 1.84*d*	14.2 14.0	39.10	4'	7.07*dd*	8.2, 1.4	129.18
4	3.65*br*, *s*		64.10	5'			130.12
6	2.82*d* 1.25*d*	11.0 11.0	53.15	6'	7.05*d*		130.90
7	1.40		25.15	1"			125.75
8	1.60 1.48		24.14	2"			135.01
9	1.45 1.40		33.50	3"	7.00*s*		110.78
10	1.84		62.00	4"			146.29
12			167.73	5"			150.04
13	5.90*d*	12.2	118.94	6"	6.95*s*		111.53
14	6.84*d*	12.2	135.78	4"-OCH_3	3.91*s*		56.71
1'			125.75	5"-OCH_3	3.95*s*		56.99

Alkaloids isolation and identification

Four alkaloids were separated by thin layer chromatography; their retention factors (R_f) were as follows: alkaloid 1, R_f = 0.392; alkaloid 2, R_f = 0.87; alkaloid 3, R_f = 0.55; and alkaloid 4, R_f= 0.35. The alkaloids were extracted of silica gel and assayed in normotensive rats and in animals that developed hypertension by angiotensin II administration. Alkaloid 3 showed high anti-hypertensive activity (Guzman et al., 2006), so it was isolated and prepared for structuralchemical identification. Spectroscopic studies identified alkaloid 3 as lythrine, with its chemical structure similar to that of the quinolizidine alkaloids group (Figure 4). The spectroscopic data of the active compound were UV (λ_{max}) 279.9; ^{1}H and ^{13}C NMR (Table 1), corresponding to those reported for lythrine (Rumalla et al., 2008). Thus, in the following experiments, alkaloid 3

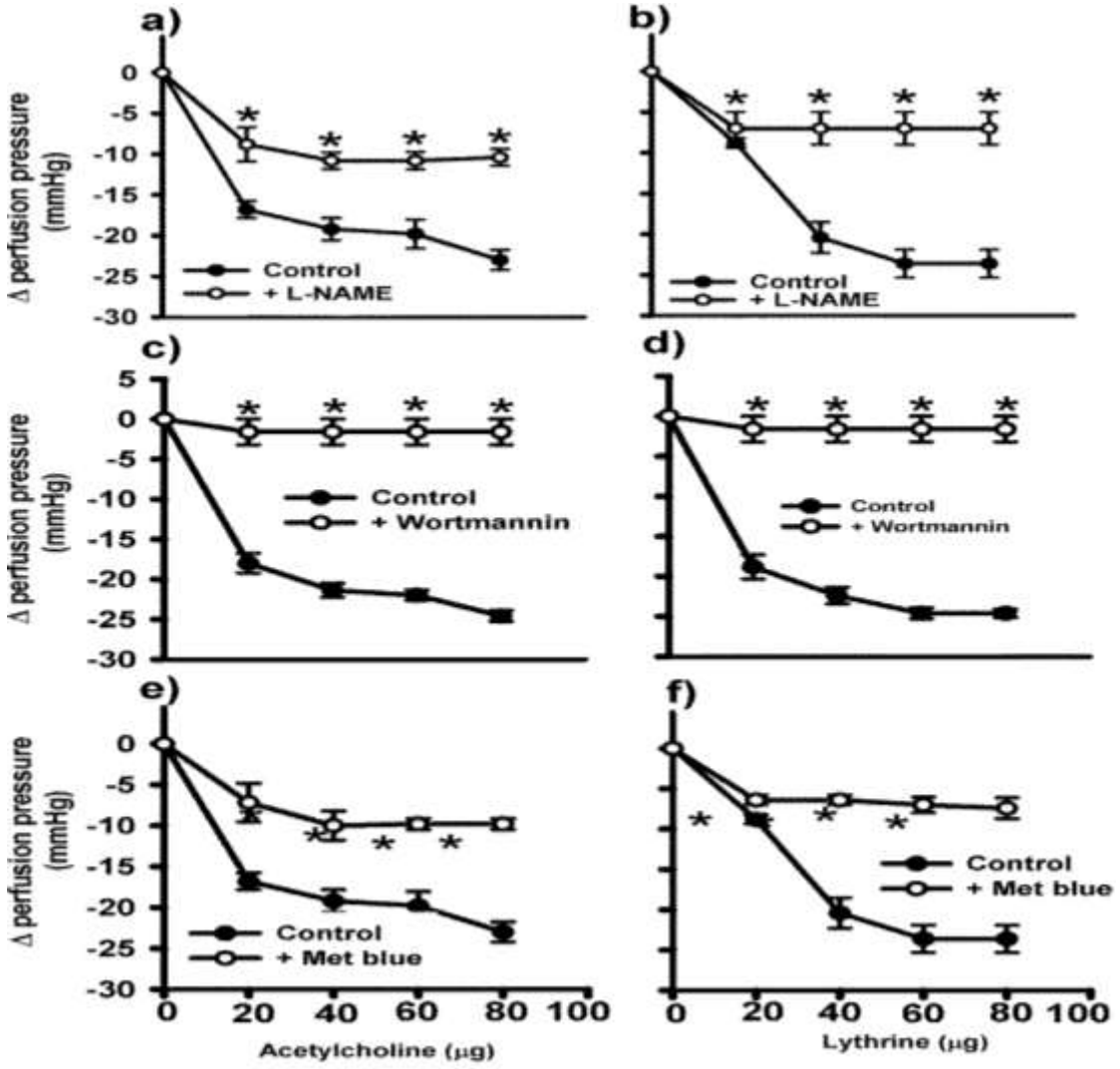

Figure 5. Effect of L-NAME (10^{-5}M) (panels a and b), wortmannin (10^{-5}M) (panels c and d), and methylene blue (10^{-5}M) (panels e and f) administration on the endothelium-dependent relaxation induced by acetylcholine (panels a, c and e) or lythrine (panels b, d and f) in phenylephrine (10^{-5}M) pre-contracted rat mesenteric vascular bed. Each point represents the mean ± S.E.M., from 6 experiments. *p< 0.05.

is referred as lythrine alkaloid.

Effect of lythrine on endothelium-derived NO-cyclic GMP pathway in MVB

A concentration-response curve for the lythrine alkaloid on the isolated and perfused MVB from normotensive rats with intact endothelium was constructed. Lythrine did not modify the baseline perfusion pressure (50±2.2 mm Hg). In Phen pre-contracted preparations, constant perfusion with lythrine (20-80 µg) produced concentration-dependent relaxation (8.8±0.6, 20.4±1.9, 23.6±1.7 and 23.6±1.7 mm Hg respectively). In all the concentration-response curves, ACh was the control drug producing similar relaxant effect as lythrine (Figures 5). When the MVB was perfused with 10^{-4} M L-NAME, the vasodilatation induced by lythrine alkaloid decreased (perfusion pressure 7±2 mm Hg, Figure 5b). A similar effect was observed with ACh (Figure 5a).

In the presence of 10^{-5} M wortmanin, the relaxant effect of lythrine (20-80 µg) was only 1.6±2 mm Hg (Figure 5d), similar to that observed with ACh (Figure 5c).

The perfusion with 10^{-5} M methylene blue decreased the relaxant effect of lythrine. The maximum relaxant effect was 7.4±1.3 mm Hg (Figure 5f), similar to that observed with ACh (Figure 5e).

These data suggest that lythrine exerts its relaxant effect on functional endothelium through the nitric oxide synthesis pathway.

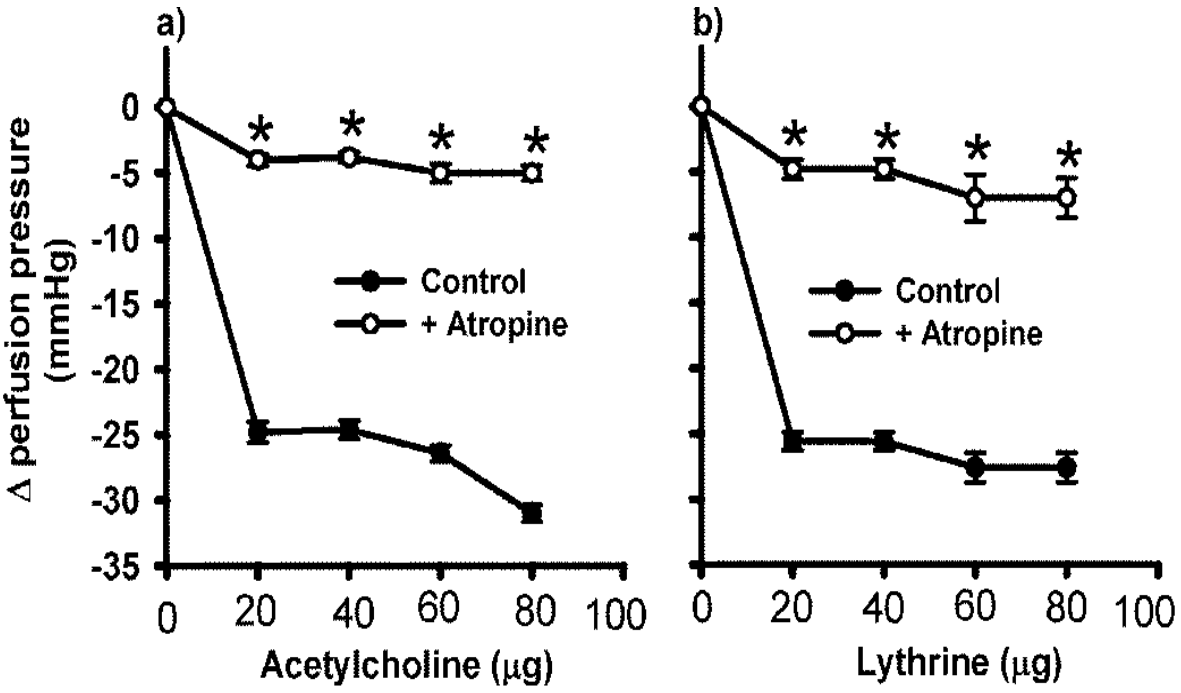

Figure 6. Effect of atropine (10^{-5}M) administration on the endothelium-dependent relaxation induced by acetylcholine (panel a) or lythrine (panel b) in phenylephrine (10^{-5}M) precontracted rat mesenteric vascular bed. Each point represents the mean ± S.E.M., from 6 experiments. *p<0.05.

Lythrine-induced vasorelaxation in MVB treated with atropine and/or diphenhydramine

In an isolated perfused MVB preparation with intact endothelium pre-contracted with Phen in the presence of 10^{-5} M atropine, the relaxant effect of lythrine (20-80 µg) was decreased (4.8±0.7, 4.8±0.7, 7±1.8, 7±1.5 mm Hg, respectively) (Figure 6b). A similar concentration-response curve was obtained with ACh (Figure 6a), suggesting that the vasodilatory effect of lythrine requires binding to a muscarinic receptor and an intact pathway of the nitric oxide synthesis.

In an isolated perfused MVB preparation pre-contracted with Phen, histamine (20-80 µg) produced a relaxant effect (18±1.5, 22±1.03, 24±0.68 and 24±0.51 mm Hg respectively) (Figure 7a). When the preparation was perfused with 10^{-5} M diphenhydramine, the histamine relaxant effect was blocked. On the other hand, the lythrine relaxant effect was not affected by diphenhydramine (Figure 7b), suggesting that the lythrine vasodilatory effect is independent of the H_1 histamine receptors.

DISCUSSION

H. salicifolia contains alkaloids with antihypertensive activity. Four alkaloids with vasorelaxant effect were obtained from *H. salicifolia* chloroform extract (HSCE). The alkaloid with the highest vasorelaxant activity, lythrine, was isolated a decade ago (Rumalla et al., 2008), but pharmacological studies on the compound have not been reported. The results suggest that the HSCE vasorelaxant effect depends on the release of nitric oxide (NO) or a NO-mediated substance. HSCE administration (10 and 20 mg/kg) to normotensive rats or animals with L-NAME-induced hypertension decreased the blood pressure. In MVB preparations with the endothelial cells eliminated by perfusion with distilled water (Criscione et al., 1984), HSCE-induced vasodilatation was significantly reduced, suggesting that it is mediated by the release of endothelium-derived substances. Vascular tone and therefore blood pressure is determined by the contractile state of vascular smooth muscle cells within the blood vessel wall. The role of endothelium is of particular importance since it regulates the vascular leiomyocytes tone by releasing potent vasoconstrictor molecules such as endothelin-1 and vasodilator substances such as NO (Godo and Shimokawa, 2017). NO is an important regulator of vascular tone and blood pressure. It has been reported that the pharmacological reduction of NO can lead to hypertension in normotensive rats (Raghavan and Dikshit, 2004; Wang et al., 2017). L-NAME administration decreases NO synthesis and develops hypertension in rats (Leo et al., 2015). In this study, animals receiving

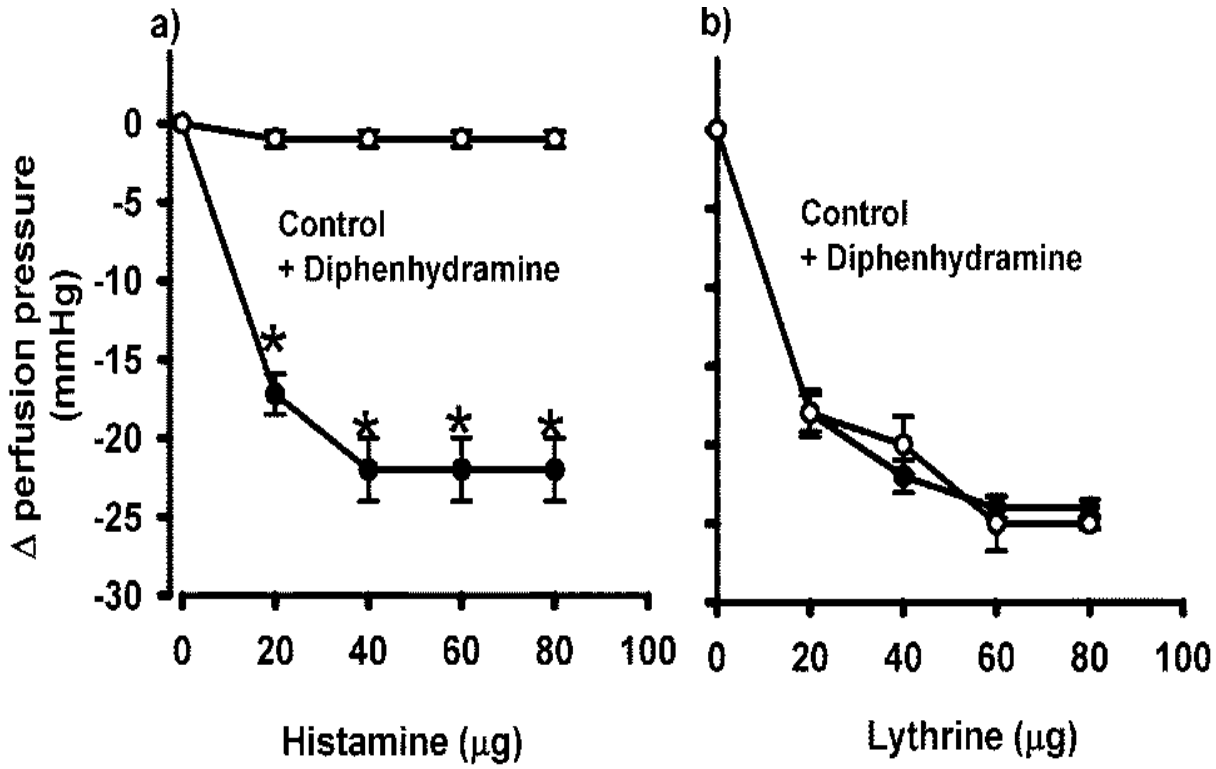

Figure 7. Effect of diphenhydramine (10^{-5}M) administration on the endothelium-dependent relaxation induced by histamine (panel a) or lythrine (panel b) in phenylephrine (10^{-5}M) precontracted rat mesenteric vascular bed. Each point represents the mean ± S.E.M., from 6 experiments. *p< 0.05.

simultaneous administration of L-NAME and HSCE showed lower systolic blood pressure as compared with animals receiving only L-NAME. Similar data were reported for *Nitraria sibirica* (Senejouxa et al., 2012), *Bidens pilosa* (Bilanda et al., 2017), *Ulmus wallichiana* (Syeda et al., 2016), *Antidesma thwaitesianum* (Kukongviriyapan et al., 2015), plants with NO-mediated hypotensive effect. The *in vitro* studies using the MVB preparation pre-contracted by Phen also support that the vasorelaxant mechanism of lythrine is likely to be mediated by activation of NO release from vascular endothelium, since the removal of endothelial cells led to absence of the response to HSCE. *H. salicifolia* exerted an important vasorelaxant effect both *in vivo* and *in vitro*. The major compound identified in HSCE was the lythrine alkaloid as its vasorelaxant effect has not been previously described. In this study, its action mechanism was elucidated using *in vitro* MVB preparations. In the preparation of isolated perfused MVB pre-contracted with phenylephrine and intact endothelium, the effect of the lythrine seemed to be endothelium dependent, as the vasorelaxant action of lythrine was abolished by L-NAME perfusion.

The involvement of cGMP in the relaxant and hypotensive effects of lythrine was verified. The soluble guanylate cyclase inhibitor, methylene blue, completely abolished lythrine -induced vasodilatation, an effect similar to that produced by *Orthosiphon stamineus* (Yam et al., 2016) and Praeruptorin A (Xua et al., 2010).

Activation of endothelial nitric oxide synthase (eNOS) depends on the formation of calcium–calmodulin complex (Su et al., 2014). However, eNOS can also be activated by phosphorylation with PI3K, a calcium-independent mechanism (Zhang et al., 2014). The PI3K-dependent activation of eNOS by wine-derived polyphenolic compounds has been reported (Ziberna et al., 2013). In the present study, the MVB was pre-contracted with phenylephrine and wortmannin, a PI3K inhibitor. Wortmannin strongly reduced lythrine-induced vasodilatation, suggesting that PI3K-dependent activation of eNOS is an important underlying mechanism in the vasodilatory effect of lythrine, similar to that reported for *Tapirira guianensis* (Rodrigues et al., 2017).

Atropine reduced lythrine-induced vasodilatation, suggesting that the vasodilatory effect of lythrine is mediated through the interaction with cholinergic muscarinic receptors. A similar action mechanism has been reported for other herbal remedies, such as *O. stamineus* (Yam et al., 2016) and *Cymbosema roseum* (Rocha et al., 2015). To verify if the effect of lythrine is mediated by muscarinic receptors with some degree of specificity, the MVB was perfused with phenylephrine

plus diphenhydramine, an H_1 receptor antagonist.

Lythrine showed vasodilatory effect both in the presence and absence of diphenhydramine, suggesting that it is mediated by muscarinic receptors and not by histamine H_1 receptors.

Conclusion

This study demonstrated that lythrine alkaloid isolated from *H. salicifolia* induces vascular relaxation in isolated MVB, which could explain its hypotensive effect in normotensive and hypertensive rats. These effects appear to be dependent on the activation of the NO/guanylate cyclase pathway. The development of pharmacological strategies capable of stimulating or maintaining endogenous NO production may contribute to improved management of several pathological conditions, such as hypertension. These findings have shown the potential of *H. salicifolia* as an herbal remedy, and contribute to providing the pharmacological basis for its use in folk medicine.

CONFLICT OF INTERESTS

The authors have not declared any conflicts of interests.

REFERENCES

Aguilar A, Camacho R, Chino S, Jacques P, López E (1994). Plantas medicinales del herbario del IMSS. Cuadros básicos para aparatos y sistemas del cuerpo humano. Instituto Mexicano del Seguro Social. Mexico pp.567. Available at: http://bases.bireme.br/cgi-bin/wxislind.exe/iah/online/?IsisScript=iah/iah.xis&src=google&base=LILACS&lang=p&nextAction=lnk&exprSearch=378665&indexSearch=ID

Baharvand-Ahmadi B, Asadi-Samani M (2017). A mini-review on the most important effective medicinal plants to treat hypertension in ethnobotanical evidence of Iran. J. Nephropharmacol. 6(1):3-8.

Balam-Ortiz E, Muñoz-Monroy OE, Rodriguez S, Barajas-Olmos F, Esquivel-Villarreal A, Huerta-Hernandez D, Ojeda-Delgado JL. (2014). Porcentaje de pacientes con hipertensión arterial esencial controlada en dos hospitales de la ciudad de Mexico. Rev. Sanid. Milit. Mex. 68(1):9-14. Available at: http://www.imbiomed.com.mx/1/1/articulos.php?method=showDetail&id_articulo=101591&id_seccion=88&id_ejemplar=9888&id_revista=16

Bilanda DC, Dzeufiet PDD, Kouakep L, Aboubakar BFO, Tedong L, Kamtchouing P, Dimo T (2017). *Bidens pilosa* ethylene acetate extract can protect against L-NAME-induced hypertension on rats. BMC Complement. Altern. Med. 17(1):479.

Blomster RN, Schwarting AE, Bobbitt JM (1964). Alkaloids of *Heimia salicifolia* I. A preliminary report. Lloydia. 27:15-24.

Byrne JA, Malone MH (1981). Protective effects of cryogenine, lythrine and certain derivatives in croton oil induced inflammation. Proc. West. Pharmacol. Soc. 24:183-187. Available at: https://www.ncbi.nlm.nih.gov/pubmed/7255446

Campos-Nonato I, Hernández-Barrera L, Rojas-Martínez R, Pedroza-Tobías A, Medina-García C, Barquera S (2013). Hypertension: prevalence, early diagnosis, control and trends in Mexican adults. Salud Publica Mex. 55(Suppl 2):S144-S150.

Go AS, Mozaffarian D, Roger VL Benjamin EJ, Berry JD, Blaha MJ, et al. (2014). Executive summary: heart disease and stroke statistics-2014 update: a report from the American Heart Association. Circulation 129(3):399-410.

Godo S, Shimokawa H (2017). Endothelial functions. Arterioscler. Thromb. Vasc. Biol. 37(9):e108-e114. Available at: https://www.ncbi.nlm.nih.gov/pubmed/28835487

Graham S (1997). Type species: *Heimia salicifolia*. Archives Kent University, Available at: http://web.archive.org/web/19970624061507/http://simon.kent.edu/Biology/Research/Shirley_Graham/Genera/heimia.html.

Guzman HE, Segura-Cobos D, De Haro R, Vazquez-Cruz B (2006). Antihypertensive effect of *Heimia salicifolia* (H.B.K) alkaloids. Pharmacology online 3:3817-3823.

Hernández-Abreu O, Torres-Piedra M, García-Jiménez S, Ibarra-Barajas M, Villalobos-Molina R, Montes S, Rembao D, Estrada-Soto S (2013). Dose-dependent antihypertensive determination and toxicological studies of tilianin isolated from *Agastache mexicana*. J. Ethnopharmacol. 146(1):187-191.

Kaplan HR, Malone M (1966). Pharmacologic study of nesodine, cryogenine, and other alkaloids of *Heimia salicifolia*. Lloydia 29:348-359.

Kukongviriyapan U, Kukongviriyapan V, Pannangpetch P, Donpunha W, Sripui J, Sae-Eaw A, Boonla O (2015). Mamao pomace extract alleviates hypertension and oxidative stress in nitric oxide deficient rats. Nutrients 7(8):6179-6194.

Lema WJ, Blankenship JW, Malone MH (1986). Prostaglandin synthetase inhibition by alkaloids of *Heimia salicifolia*. J. Ethnopharmacol. 15(2):161-167.

Leo MD, Kandasamy K, Subramani J, Tandan SK, Kumar D (2015). Involvement of inducible nitric oxide synthase and dimethyl arginine dimethylamino hydrolase in N_{ω}-Nitro-L-arginine methyl ester (L-NAME)-induced hypertension. Cardiovasc. Pathol. 24(1):49-55.

Luna-Vázquez FJ, Ibarra-Alvarado C, Rojas-Molina A, Rojas-Molina I, Zavala-Sánchez MA (2013). Vasodilator compounds derived from plants and their mechanisms of action. Molecules 18(5):5814-5857.

Magos-Guerrero GA, Santiago-Mejía J, Carrasco OF (2017). Exploratory studies of some Mexican medicinal plants: cardiovascular effects in rats with and without hypertension. J. Intercult. Ethnopharmacol. 6(3):274-279.

Malone MH, Rother A (1994). Heimia salicifolia: a phytochemical and phytopharmacologic review. J. Ethnopharmacol. 42(3):135-159.

Raghavan SA, Dikshit M (2004). Vascular regulation by the L-arginine metabolites, nitric oxide and agmatine. Pharmacol. Res. 49(5):397-414.

Rocha BA, Barroso-Neto IL, Teixeira CS, Santiago MQ, Pires AF, Souza LA, Nascimento KS, Sampaio AH, Delatorre P, Assreuy AM, Cavada BS (2015). CRLI induces vascular smooth muscle relaxation and suggests a dual mechanism of eNOS activation by legume lectins via muscarinic receptors and shear stress. Arch. Biochem. Biophys. 565:32-39.

Rodrigues AM, Guimarães DO, Konno TU, Tinoco LW, Barth T, Aguiar FA, Lopes NP, Leal IC, Raimundo JM, Muzitano MF (2017). Phytochemical study of *Tapirira guianensis* leaves guided by vasodilatory and antioxidant activities. Molecules 22(2):E304.

Rumalla CS, Jadhav AN, Smillie T, Fronczek FR, Khan IA (2008). Alkaloids from *Heimia salicifolia*. Phytochemistry 69(8):1756-1762.

Senejouxa F, Girarda C, Aisab HA, Bakrib M, Kerramc P, Berthelota A, Bévalota F, Demougeota C (2012). Vasorelaxant and hypotensive effects of a hydroalcoholic extract from the fruits of *Nitraria sibirica* Pall. (Nitrariaceae). J. Ethnopharmacol. 141:629-634.

Sousa GJ, Oliveira PWC, Nogueira BV, Melo AF Junior, Faria TO, Meira EF, Mill JG, Bissoli NS, Marcelo P (2017). Fructose intake exacerbates the contractile response elicited by norepinephrine in mesenteric vascular bed of rats via increased endothelial prostanoids. J. Nutr. Biochem. 48:21-28.

Su KH, Lin SJ, Wei J, Lee KI, Zhao JF, Shyue SK, Lee TS (2014). The essential role of transient receptor potential vanilloid 1 in simvastatin-induced activation of endothelial nitric oxide synthase and angiogenesis. Acta. Physiol. 212(3):191-204.

Syeda AA, Lahiria S, Mohanb D, Valicherla GR, Gupta AP, Riyazuddin M, Kumar S, Maurya R, Hanif K, Gayen JR (2016). Evaluation of anti-hypertensive activity of *Ulmus wallichiana* extract and fraction in SHR, DOCA-salt- and L-NAME-induced hypertensive rats. J.

Ethnopharmacol. 193:555-565.
Tirapelli CR, Ambrosio SR, de Oliveira AM, Tostes RC (2010). Hypotensive action of naturally occurring diterpenes: a therapeutic promise for the treatment of hypertension**.** Fitoterapia. 81(7):690-702.
Wang Y, Zhang F, Liu Y, Yin S, Pang X, Li Z, Wei Z (2017). Nebivolol alleviates aortic remodeling through eNOS upregulation and inhibition of oxidative stress in L-NAME-induced hypertensive rats. Clin. Exp. Hypertens. 39(7):628-639.
World Health Organization (WHO) (2013). A global brief on hypertension: silent killer, global public health crisis. Document number: WHO/DCO/WHD/2013.2. pp.1-40.
Xua Z, Wang X, Dai Y, Kong L, Wang F, Xu H, Lu D, Song J, Hou Z (2010). (+/-)-Praeruptorin A enantiomers exert distinct relaxant effects on isolated rat aorta rings dependent on endothelium and nitric oxide synthesis. Chem. Biol. Interact. 186(2):239-246.
Yam MF, Tan CS, Ahmad M, Shibao R (2016). Vasorelaxant action of the chloroform fraction of Orthosiphon stamineus via NO/cGMP pathway, potassium and calcium channels. Am. J. Chin. Med. 44(7):1413-1439.
Zhang W, Han Y, Meng G, Bai W, Xie L, Lu H, Shao Y, Wei L, Pan S, Zhou S, Chen Q, Ferro A, Ji Y (2014). Direct renin inhibition with aliskiren protects against myocardial ischemia/reperfusion injury by activating nitric oxide synthase signaling in spontaneously hypertensive rats. J. Am. Heart. Assoc. 3(1):e000606.
Ziberna L, Kim JH, Auger C, Passamonti S, Schini-Kerth V (2013). Role of endothelial cell membrane transport in red wine polyphenols-induced coronary vasorelaxation: involvement of bilitranslocase. Food Funct. 4(10):1452-1456.

African Journal of Pharmacy and Pharmacology